A COMPARATIVE STUDY OF CHLOROPHYLL RENEWAL IN VARIOUS PARTS OF THE PLANT

A. A. Shlyk, V. I. Gaponenko, I. V. Prudnikova, T. V. Kukhtenko, Ya. P. Lyakhnovich, and V. L. Kaler

Laboratory of Biophysics and Isotopes, Academy of Sciences of the Belorussian SSR, Minsk

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In studying the chlorophyll renewal process in plants we obtained data of a preliminary character which indicated the differentiated character of the destruction of the pigment-renewing molecules [1]. The experimental results indicated the possibility that the likelihood of the chlorophyll molecule changes with age. Turchin [2] obtained indications of a similar selectivity in the dark decomposition of protein and chlorophyll. However, in neither case was it clear whether this discrimination occurs in the decomposition of the pigment molecules (possibly the whole complex of chlorophyll-protein) in the same chloroplast, or is the result of selective decomposition of older chloroplasts, related to the age of cells. The important stage in deciding this question is the comparison of the chlorophyll renewal course in different parts of the plant, which becomes necessary in evaluating the correctness of investigation usually conducted of the renewal process in the plant as a whole. In the present study the first data of this type are published.

METHODS

The aquatic hornwort Ceratophyllum demersum L., grown in a river under natural conditions, was used for study. Five hundred young shoots were selected for the experiment, as far as possible equal in length and free from side branches and buds. After keeping the plants in the laboratory for two weeks in periodically changed tap water, they were transferred for 11 hours to a bath with 20 liters of water containing 30 mC of C^{14} in the form of Na_2CO_3 plus $NaHCO_3$. The bath was illuminated by 16 incandescent 600-watt lamps, which together with the daylight created a luminosity on the water surface of the order of 5000 lux. The bath contents were stirred periodically in the process of exposure. Then the plants were carried over to a second bath without C^{14}, in which they were washed free of the mechanically absorbed liquid of the first bath by a 7-fold change of a large volume of water (about 250 liters).

The shoots were kept in the second bath for one and a half months on tap water, which was changed twice a day in order to prevent repeated assimilation of C^{14} liberated by the plants. The illumination of this bath was achieved in the same manner as the previous one, with a daily illumination period of thirteen hours (from 9 A.M. to 10 P.M.).

Systematically, after 1-2 days (at most 3-5) at ten o'clock in the morning twenty shoots were taken from the bath and fixated by hot steam. Each shoot was cut into three parts, the upper, the middle, and the lower, so that three fractions were obtained from each sample. Dried at 35 to 40° in a current of warm air, the samples then were preserved in a refrigerator (wrapped in tracing cloth packages and placed in a desiccator).

To eliminate the effects of systematic errors on the course of each kinetic curve, the samples were analyzed in random order. The analysis was begun by weighing the given fraction of the sample and determining the specific activity of the growing tissue by compression, with subsequent measurement of radioactivity in a continuous thick layer [3]. Then the material was ground in a mortar with addition of granular $CaCO_3$ in the presence of 80% acetone. The extract was transferred quantitatively onto a glass filter, after which an aliquot portion (about 1 ml) was taken for spectrophotometry by an SF-4 or SF-5 spectrophotometer, and the contents of chlorophyll a, chlorophyll b, and carotenoids were calculated [4]. Pigments of the main portion of the extract (100-150 ml) were transferred to ether with multiple water washings, thus removing acetone and water-soluble substances extracted from the plants.

In view of our previous data [5] on the difficulty of separating C^{14}-labeled admixtures from chlorophyll, purification of chlorophylls a and b was conducted by fivefold paper chromatography in solvent mixtures [6] (changed each time) similar to those suggested by Bauer [7] and Sapozhnikov and co-workers [8]. The specific radioactivity of the carbon of the pigments was determined in a continuous thin layer [9] with subsequent spectrophotometry of the ethereal solution of the pigment [10]. Determination of the specific activity was

usually repeated two or three times to prevent considerable experimental error. In the experiments only the data characterizing separate portions of plants were obtained directly. However results relating to the whole shoot were found by appropriate calculation, taking into account the weight of each fraction.

RESULTS

The State of the Plant and the Pigment System

Data obtained as a result of weighing the experimental samples are depicted in Fig. 1, recalculated per single shoot. As components of the total weight of the shoot, the weight of separate portions is shown: the lower the middle, and the upper. Even though the sample of 20 shoots apparently was not selected completely uniformly, and at times contained either smaller or larger shoots, the over-all data clearly denote the tendency (shown by the straight line) to a gradual increase in the weight of the shoots. Consequently, during the entire experimental period there was a slow growth of the plants, with a daily mean average growth increment of 0.3% of the plant's initial magnitude.

To facilitate the analysis of radioactivity curves, the experiment was conducted in the second half of the summer, after the plants flowered, and during its course a constant destruction of the plants' pigments occurred, but the changes in the content of each pigment in the whole plant (Fig. 2, A) were dependent on the decrease of its concentration in each portion (Fig. 2, B). Comparison of these data with the pigment concentration in the aquatic plant located in the river shows that until the very last period it was higher in the experimental shoots. Within the range of accurate determination it is most proper to represent the character of destruction by straight lines in noting the general direction of the process, without indicating whether the process is either accelerated or retarded by the end of the experiment. In Fig. 2, B data characterizing concentrations of chlorophylls a and b in individual shoot portions are marked by various points, while the decline of concentration in the entire shoot is shown by straight lines. Insofar as the velocity of pigment destruction is concerned (Table 1), no essential differences between the shoot portions were found, although in the middle portion the chlorophyll a concentration apparently declines at a slower rate.

It is very important to note that the destruction of chlorophylls a and b generally proceeds similarly, so that the ratio of both pigments (Fig. 2, B) remained constant at all times (2.15). In the upper shoot portions it was somewhat higher (2.3) than in the lower ones (2.0). Apparently, carotenoids disappeared together with chlorophyll, so that an approximate constant was preserved in the ratio of green and yellow pigments: on the average, 4.5 per shoot. Consequently, all plastid pigments were destroyed simultaneously, as happens in the decomposition of the chloroplast as a whole. This is important because with such characteristics the decomposition will not be reflected in the ratio of radioactivity curves unless some kind of discrimination exists between pigment molecules, chloroplasts, etc.

Specific Radioactivity of Carbon in Chlorophylls A and B

In studying the renewal processes the character of variations in specific activity is most significant. The curves indicating its dependence on the time elapsed since the plant's assimilation of C^{14} are shown in Fig. 3 for carbon of a and b chlorophylls in the entire shoot. However, the analysis of changes in the pigment's specific activity in individual fractions shows that this picture is not characteristic for all of the shoot's portions (Fig. 4). Only in the case of chlorophyll a do all the curves pass through a maximum. When the maximum is calculated for the whole plant at about the 13th day, then in different fractions they are found at about the 7th, 10th, and 17th day correspondingly for the upper, middle, and lower portions. At the same time the steepness of increases and decreases in specific activity lessens, and so does the expressed maximum, which is quite diffuse in the lower fraction, so that it even becomes difficult to locate it.

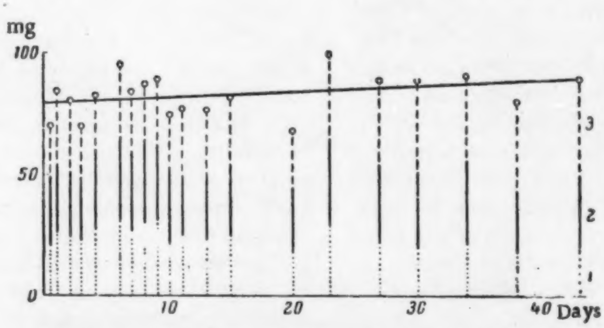

Fig. 1. Weight of one experimental shoot and its parts. 1) lower portion; 2) middle portion; 3) upper portion.

TABLE 1. Velocity of Pigment Decomposition in Experimental Shoots

shoot portion	quantity of pigment decomposed in 24 hours (in % of the initial)		
	chlorophyll $\underline{a}$	chlorophyll $\underline{b}$	carotenoids
upper	1.7	1.6	1.6
middle	1.4	1.6	1.4
lower	1.7	1.7	1.4
entire shoot	1.5	1.6	1.5

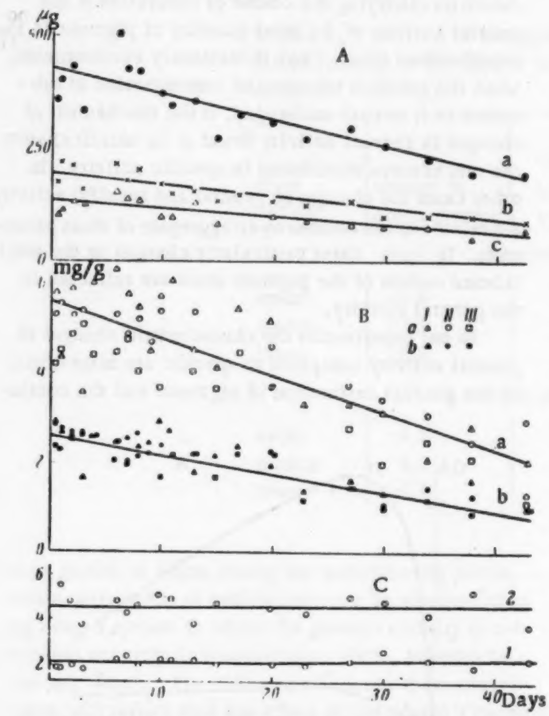

Fig. 2. Change in the plant pigment content: A) chlorophyll $\underline{a}$ content (curve a), chlorophyll $\underline{b}$ (curve b), and carotenoids (curve c) in one shoot (in μg); B) concentration of chlorophyll $\underline{a}$ (curve a) and chlorophyll phyll $\underline{b}$ (curve b) in the shoot (straight lines) and in its upper (I), middle (II), and lower (III) portions (in mg per g of tissue); C) ratio of pigment content: 1) chlorophylls a/b; 2) chlorophylls (a + b)/carotenoids.

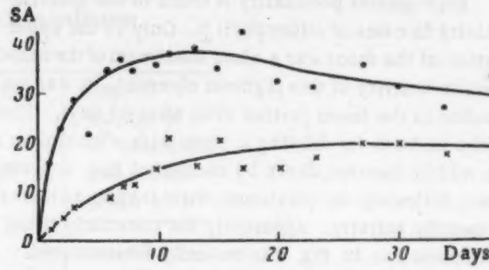

Fig. 3. The relation of specific activities (SA, pulses/min·μg C) of carbon in chlorophyll $\underline{a}$ (a) and chlorophyll $\underline{b}$ (b) in the aquatic plant shoot to the time elapsed since the plant assimilated C^{14}.

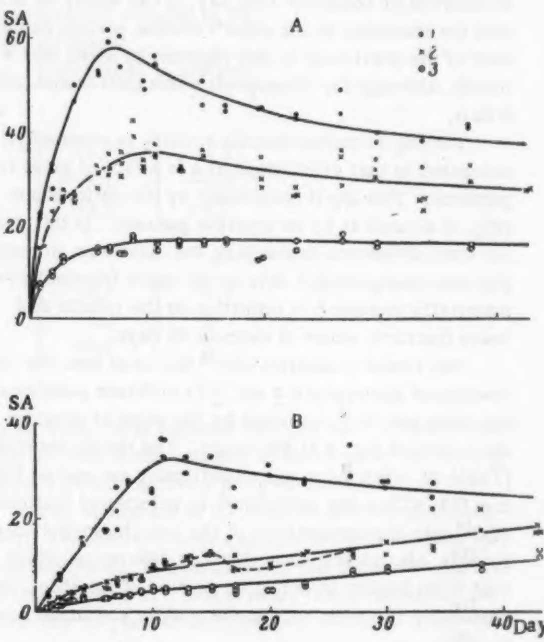

Fig. 4. Variation in specific activities (SA, pulses/min·μg C) of carbon in chlorophyll $\underline{a}$ (A) and chlorophyll $\underline{b}$ (B) in various portions of the shoot: upper (1), middle (2), and lower (3).

Even greater peculiarity is found in the specific activity in cases of chlorophyll b. Only in the upper portion of the shoot was a clear maximum of the carbon's specific activity of this pigment observed. It was not reached in the lower portion even after 43 days. There is also no basis for drawing a curve with a maximum for the middle fraction, shown by the dotted line, especially since, following the maximum, there is again an increase in specific activity. Apparently the constantly rising continuous line in Fig. 4 is basically substantiated.

Nonetheless, since after a reasonable period of time the specific activity of any substance participating in the metabolic process must approach zero for general reasons, the existence of maximums on the last two curves also cannot be doubted. Consequently the obtained data indicate that these maximums are located outside the limits of the experiment and are achieved after the 43rd day. If one takes into account that in the shoot's upper portion the maximum of chlorophyll b specific activity is observed at about the 10th day, it can easily be seen that the transition to the shoot's middle portion causes a shift of the maximum in this pigment by more than a month, although for chlorophyll a this shift lasted only 3 days.

The lag in carbon specific activity in chlorophyll b compared to that of chlorophyll a is a fact of great importance. This lag is manifested by the entire shoot (Fig. 3) as well as by its separate portions. In this case the time difference in reaching the maximum in both pigments (comprising 3 days in the upper fraction) is very materially increased in transition to the middle and lower fractions, where it exceeds 26 days.

The initial velocities of C^{14} inclusion into the composition of chlorophylls a and b in different portions of the shoot can be determined by the slope of tangents to the curves of Fig. 4 at the origin. The results obtained (Table 2), while once more confirming our earlier findings [11, 12] on the differences in velocity of inclusion of C^{14} into the composition of the two chlorophyll components, also clearly show that this difference, which is very considerable in the upper portion of the shoot, increases by $1\frac{1}{2}$ times both in the middle and in the lower portion.

Consistent with the exponential character of the decrease in specific activity following the maximums, the half-life (in days) and the constant of the decrease, equal to the portion of the isotope extracted from a given stock of pigment, can be calculated for 24 hours from a plot on a semilog scale. In the case of chlorophyll a these magnitudes were found to be respectively 41 and 0.017 for the upper, 57 and 0.012 for the middle, and 180 and 0.004 for the lower portion of the shoot. Since the specific activity maximum of chlorophyll b was attained in the upper fraction only, the half-life and decomposition constant can only be calculated in this case. The magnitudes found — 53 and 0.013 — indicate that the changes have a slower character than is the case for chlorophyll a in the same portion of the shoot.

Thus, in chlorophyll a and especially in chlorophyll b the transition to the older portions of the shoot is accompanied by a lesser expression of all evaluable characteristics of the process: Velocities of the increase and decrease of specific activity are lessened, the maximum is reduced, and the time needed for reaching it is increased. Similar changes are characteristic in comparing chlorophyll b with chlorophyll a.

General Radioactivity of Chlorophylls A and B

Apart from the specific activity, the essential datum for clarifying the course of restoration is the general activity of the total quantity of pigment in the experimental item. Only in stationary environments, when the system's volume and concentration of substance in it remain unchanged, is the mechanism of changes in general activity found to be identical with the mechanisms manifested in specific activity. In other cases the changes in general and specific activity are related to one another by an aggregate of these parameters. In such cases particularly, changes in the total labeled carbon of the pigment stock are reflected in the general activity.

In our experiments the characteristic changes in general activity compared to specific are determined by the gradual destruction of pigments and the contin-

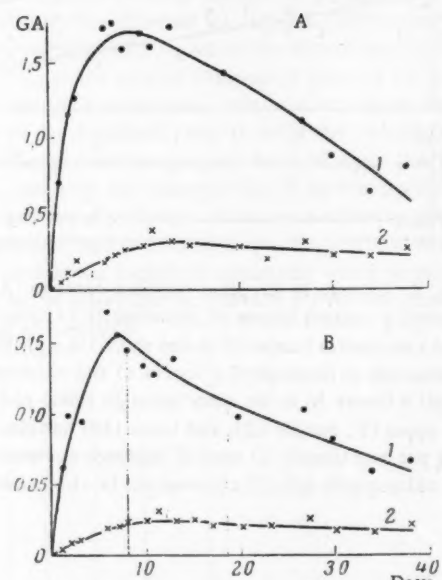

Fig. 5. Relation of general activity (GA, μC) of chlorophyll a (1) and chlorophyll b (2) per g of tissue (A) and in the shoot (B) to time elapsed after C^{14} assimilation.

TABLE 2. Initial Velocities of C^{14} Incorporation into the Composition of Chlorophylls a and b in Different Portions of Hornwort

shoot portion	velocity of C^{14} inclusion, pulses/min·μg C·day		ratio of velocities of C^{14} inclusion into chlorophylls a + b
	chlorophyll a	chlorophyll b	
upper	35	4.2	8
middle	23	2.0	12
lower	15	0.9	17

TABLE 3. Velocities of Changes Occurring in Pigments in Different Portions of the Shoot (in % of magnitudes observed on about the 20th day)

shoot portion	change in concentration	change in specific activity	change in pigment's general activity in 1 g of tissue	
			calculated	observed
		chlorophyll a		
upper	−2.6	−1.7	−4.3	−4.4
middle	−1.9	−1.2	−3.1	−2.9
lower	−2.6	−0.4	−3.0	−3.2
		chlorophyll b		
upper	−2.3	−1.3	−3.6	−3.8
middle	−2.3	+2.0	−0.3	0
lower	−2.6	+3.1	+0.5	0

uous growth of shoots during the experimental period. Some segregation of both factors can be accomplished by using a system in which the general activity is calculated not only for the shoots as a whole, but also for the unit weight. The difference between these magnitudes will reflect first the effect of the system's increase in volume at the expense of growth, while by comparing the course of curves of specific and general activity in the tissue unit, the value of changes in concentration will predominate.

As is shown in Fig. 5, the general activity of chlorophyll a rises steeply during the initial days of the experiment and then begins to drop fairly rapidly. The velocity of this drop consists of the velocities of the decrease of specific activity and the changes in the pigment content. Thus, on about the 20th day of the experiment the general activity of chlorophyll a per gram of tissue decreased in one day by 3.1%, and in the whole shoot by 2.7%. The difference in these magnitudes is quite close to the growth rate of the shoots (0.3%). The average concentration of chlorophyll a in the shoot dropped during this period by 2.0%, the specific activity by 1.4%. It is apparent that the sum of both velocities is close to the velocity of the decrease in the pigment's general activity per g of tissue.

The maximum in the general activity of chlorophyll b is observed later and at a lower level than for chlorophyll a. The decrease in this pigment's general activity occurs at a slower rate. Thus, on about the 20th day its general acitivity per gram of tissue decreased by 1.5% daily, and in the whole shoot by 1.1%. The cause of this delayed action is the more protracted increase in specific activity, which on about the 20th day still continued to grow by 0.9% daily. If one discounts the decrease in chlorophyll b concentration by 2.4%, the coincidence is apparent between the magnitudes of values of the decrease of general activity velocities, calculated as an algebraic sum, and the ones actually observed. The difference in velocities of the decrease of chlorophyll b general activity in 1 g of tissue and in the whole shoot is also dependent on the growth of the plant.

In calculating the general activity of chlorophyll in 1 g of tissue a comparison can also be made between

different portions of the shoot in this relation. Such curves are presented in Fig. 6. In Table 3 a comparison is presented of velocities of different chlorophyll changes in individual portions of the shoot, similar to the one just conducted for an undivided shoot. If one takes into consideration the great inaccuracy in determining the magnitudes comprising the diagram, then the agreement obtained between the calculated and the observed values is fully satisfactory and affirms the internal consistency of the data. It should also be noted that such simplified calculations, which bring together the effects of several interrelated processes merely to an algebraic sum of their velocities, naturally could not produce a total coincidence of calculated and observed results.

In comparing Fig. 6 with Fig. 4 numerous features are noted in curves of pigment general activity per g of tissue compared with curves of specific activity. The curves drop more steeply, reflecting the effect of declining concentration. At the same time the maxima in both types of curves are observed in the same circumstances, while two curves in Fig. 6, changing to horizontal lines, correspond to the ascending curves of Fig. 4. Apparently in these cases the specific activity uniquely correlates with the pigment destruction.

The general activity of chlorophyll b rises even more slowly compared to chlorophyll a than was observed in the study of specific activity. And while there is no systematic displacement in the maximum of the general activity of chlorophyll a in Fig. 6 in relation to transition to the older parts of the shoot, as previously, there is a clear expression here of the lateness of the maximum for chlorophyll b compared to chlorophyll a.

It is characteristic of the general activity as well as the specific activity that the initial growth velocity decreases in proportion to the transition from the upper portion of the shoot to the middle, and more so to the lower. From Table 3 one can also conclude that in general the relative velocity of the drop in general activity decreased in the same direction. Some inversion of experimentally observed magnitudes for chlorophyll a in the middle portion compared to the lower one, leading to the possibility of intersection of the middle curve with the upper one, is conditioned by a small decrease of pigment concentration in the middle of the shoot and does not contradict this conclusion.

Relative Radioactivity of Carbon in Chlorophylls A and B

The analysis of chlorophyll radioactivity curves requires a simultaneous evaluation of C^{14} behavior in the whole growing tissue. Figure 7, A shows the change in general activity of one shoot in various samples, dependent on gradual withdrawal of the isotope in the metabolic process. It has been shown by numerous radiobiological studies [13] that although the isotope portion withdrawn in unit time steadily decreases, the typical withdrawal curve is close to exponential. Therefore, we present data on the special activity of the portions of shoots in Fig. 7, B on a semilogarithmic scale in the form of straight lines without accounting for the

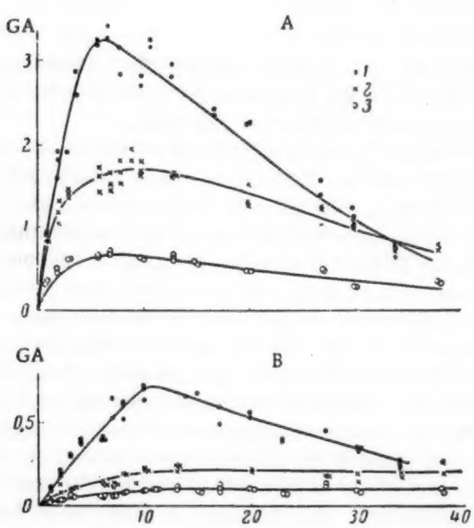

Fig. 6. Changes in general activity (GA, μC) of chlorophyll a (A) and chlorophyll b (B) per g of tissue in different parts of the shoot: upper (1), middle (2), and lower (3).

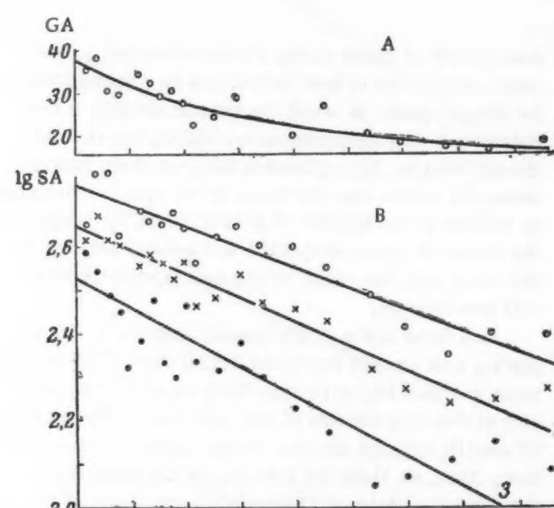

Fig. 7. Changes of C^{14} content in the plant: A) General radioactivity (GA) of the shoot (in μC); B) logarithm of specific activity (SA) of the tissue (μC/g); portions of the shoot: 1) upper, 2) middle, 3) lower.

gradual decrease of the withdrawal constant, which is especially perceptible in the lower fraction. Calculated by straight lines the period of C^{14} half-life withdrawal from upper shoot portions was 31 days, from middle portions 27 days,* and from lower, 22 days. Correspondingly, the isotope portion withdrawn in 24 hours from the upper fraction was 0.022; from the middle, 0.026; from the lower, 0.032. Consequently, the older portions of the shoot released the labeled carbon faster than the young ones.

Figure 7,B shows that in the beginning of the experiment, i.e., immediately after the end of photosynthesis in presence of the isotope, the C^{14} is especially abundant in the younger portions of the plant. As a result of differences in half-life withdrawal periods, this correlation was not only maintained but emphasized with time. This deserves consideration in the analysis of the chlorophyll's radioactivity curves. In particular, this one fact of a decrease in the plant's tissue radioactivity while in transit from the upper

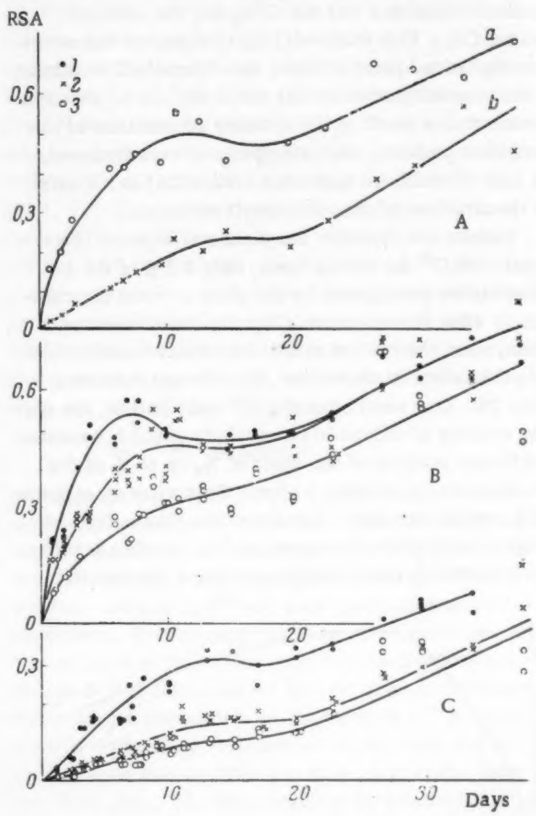

Fig. 8. Relative specific activity (RSA) of chlorophylls. A) Ratio of carbon specific activity in chlorophyll a (a) and chlorophyll b (b) in the shoot to the specific activity of the shoot's carbon; B and C) ratio of specific activity of chlorophyll a carbon (B) and chlorophyll b (C) to the specific activity of carbon in different portions of the shoot: upper (1), middle (2), and lower (3).

portion of the shoot to the lower is sufficient to explain the different levels of chlorophyll radioactivity in different portions of the shoot irrespective of some metabolic features of the pigment itself. However, this holds true only on qualitative comparison.

For a quantitative comparison we recalculated the specific activity of tissues in different portions of the shoot, accounting for a definite content of carbon in dried samples by special analyses and we related the specific activity of carbon of chlorophylls a and b to the obtained magnitudes at the same time. The curves obtained in this manner, of changes in pigment's relative specific activity during the period of the experiment, are presented in Fig. 8.

It is evident that the radioactivity level of the tissue carbon plays an important role in determining the radioactivity level of pigments, and therefore the general type of relationship is similar in many cases. Possibly a decrease of tissue activity determines the rise of the right-hand portion of all curves of chlorophyll's relative activity. However, the complex form of curves describing the specific activity maximum indicates the absence of a simple relationship between the compared parameters and shows that a direct correlation between them does not account for all the observed phenomena. In particular, the above noted difference in velocities of accelerated specific activity in different portions of the shoot cannot be ascribed only to the differences in radioactivity of carbon in tissue. Like all specific and general radioactivity, the relative specific activity of a and b chlorophylls increases most rapidly in the upper portion of the shoot, more slowly in the middle, and slowest in the lower portion. The slope of the curves nearest the origin for the lower portion of the shoot is $^1/_3$ that of the slope for the upper portion, while the slope for the middle fraction is intermediate. However, as might be expected, the above noted difference in velocity of incorporation of labeled carbon into the composition of chlorophyll a compared to chlorophyll b still holds true. Slopes of the curves relating to chlorophyll a are of a higher order of magnitude than those of curves of relative specific activity of chlorophyll b.

At the end of the experiment relatively little C^{14} remained in the tissue, and errors in measurement of radioactivity are very prominent in values obtained from experimental work. Therefore, the considerable scattering of points on the right side of Fig. 8 is under-

* A portion of the data on specific activity of tissue in the middle of the shoot was not obtained by direct measurements, but as a result of interpolation between the upper and lower fractions. Aside from the last points they all fit satisfactorily together with the experimental points in one straight line. However, discounting the interpolated points, the half-life withdrawal period would have taken 28 days.

standable. This makes it difficult to fit the curves accurately to the later points, and accordingly leaves unclear the significance of the convergence of the curves on the figure in that portion where the determining factor is a decrease in tissue activity.

However, as a result of a decrease in pigment concentration, the growth of relative specific activity does not produce in late stages of the experiment any increase in the portion of C^{14} of tissue entering the chlorophyll a composition, and only in chlorophyll b does the ratio of the pigment's general activity to the shoot's general activity (Fig. 9) show a gradual constant increase. This indicates that in the conditions of pigment destruction taking place in the experiment, the loss of C^{14} from the plant's store of chlorophyll a molecules proceeds faster, while from the store of chlorophyll b molecules it is slower than from the growing tissue as a whole. Thus, Fig. 9 once again indicates a slower change in radioactivity of chlorophyll b than that of chlorophyll a.

DISCUSSION OF RESULTS

The incorporation of C^{14} into chlorophyll, observed simultaneously with the decomposition of pigments, is only possible in the renewal process. Consequently, the obtained data once again indicate its existence. In such conditions the rate of renewal, determined by the slowest of two reactions—synthesis and decomposition—equals the rate of acceleration in the relative specific activity of the studied substance, calculated in relation to its direct predecessor. At the present time the determination of these values for chlorophyll is still impossible. Apart from experimental difficulties, this is due to the incompleteness of our knowledge of the nature of the immediate predecessor of chlorophyll in green leaves, which would have to be identified, apparently as protochlorophyll or chlorophyllid. The isolation of its remote antecedents, together with chlorophyll, remains a complex matter.

However, we assume that for comparing renewal velocities in various portions of the shoot a sufficiently characteristic value is the ratio between the specific activity of chlorophyll carbon and tissue carbon. Apparently, at similar velocities of metabolism in different portions of the shoot, the specific activity of pigment carbon in each of them would have been proportional to the tissue specific activity, depending on the initially introduced quantity of the isotope. Consequently, the data obtained in the present study on various curves of the relative specific activity of chlorophyll a, as well as chlorophyll b, in separate portions of the shoot indicate various velocities of metabolism. The slope of these curves shows that the velocity of the over-all metabolic process leading to the incorporation into chlorophyll of C^{14} assimilated by the plant is approximately 3 times larger in the upper and $1\frac{1}{2}$ times larger in the middle portion than in the lower.

The importance of this conclusion consists in the fact that it indicates how strongly the age of tissue in individual sectors of the same plant, and even the shoot, affects the inclusion of labeled carbon into chlorophyll, and indicates the necessity of a differentiated approach to the study of such renewal phenomena as would appear to be distorted in a more generalized study. In subsequent investigations it should be established whether the observed differences in velocities of the over-allmetabolic process include the differences in final or previous stages.

The obtained results also bring out some other conclusions. A sufficiently protracted acceleration of the specific, general and relative radioactivity of chlorophylls a and b in the beginning of the experiment indicates the considerable length of the path traveled by labeled carbon after its assimilation until its incorporation into chlorophyll. This acceleration we observed even after the plant was removed from the labeled medium, i. e., under conditions when the photosynthetically assimilated substance was not $C^{14}O_2$ but the ordinary natural CO_2. This indicates [14] that, despite the occasionally voiced point of view, the chlorophyll formation in the renewal process occurs not in the act of photosynthesis but as a result of the ordinary metabolism of assimilation products, and principally of carbohydrates, the role of which we indicated earlier [15] as material for the structure of the chlorophyll molecule.

Despite the fact that the plant was exposed in a vessel with C^{14} for eleven hours, only 0.1% of the labeled carbon assimilated by the plant entered the chlorophyll after that exposure (Fig. 9). Even after two weeks, when the portion of C^{14} of the shoot found in chlorophyll reached its maximum, this amount remained below 1%. One week after the C^{14} assimilation, the specific activity of carbon in chlorophylls a and b consisted in different portions of the shoot of $1/20$ up to $1/2$ of the specific activity of tissue carbon. Even after an exposure of $1\frac{1}{2}$ months this ratio remained less than unity, although it continually increased, and the specific activity of chlorophyll carbon closely approached the specific

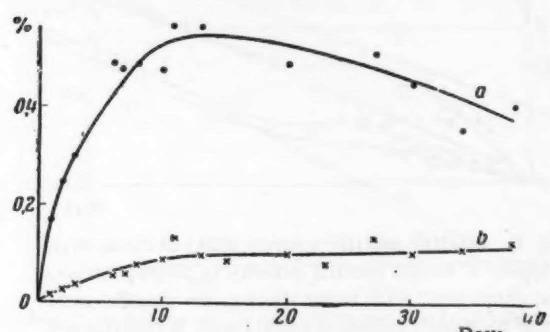

Fig. 9. Portion (in %) of radioactive carbon of the shoot entering into the composition of chlorophyll a (a) and chlorophyll b (b).

activity of tissue carbon. These data are in harmony with the consideration of chlorophyll as a rather conservative substance, involved at a considerably slower pace than many other compounds in the processes of plant metabolism. This conclusion shows again that chlorophyll cannot by any means be considered among the first photosynthetic products or substances close to them, as is sometimes done (for greater particulars see [14]).

Analyzing the concrete results of the present study, we noted that all the curves characterizing radioactivity of chlorophyll b rise more slowly, and that the maximums are reached later than in chlorophyll a. The difference between the shoot portions in the case of chlorophyll b is also especially marked. The lesser velocity of C^{14} inclusion into chlorophyll b compared with chlorophyll a indicates that the path traveled by carbon assimilated by the plant until its incorporation in one chlorophyll component is longer than in the other.

In a series of studies we considered the biosynthetic relationships of chlorophylls a and b in the renewal process, without deciding whether there is subsequent formation of chlorophyll b through chlorophyll a or parallel formation of both pigments from a common antecedent. The above-cited data favor a longer biosynthetic chlorophyll b chain than for chlorophyll a. However, the correlation, in the present study, of curves of the specific activity of both pigments in the upper portion of the shoot, with the maximum of chlorophyll b under the curve of the specific activity of chlorophyll a, is in keeping with the concept of a precursor and a product, if only one admits a metabolic heterogeneity of chlorophyll a, with an existence of some portion of it in a form supplementary to the biosynthetic chain. A detailed discussion of this problem is beyond the scope of this study and will be done separately.

SUMMARY

Specific and total radioactivity curves have been obtained for carbon in chlorophylls a and b as well as the ratio of these activities to the specific and total activities of carbon in the tissues of hornwort placed in a medium containing C^{14} and then transferred to natural conditions. The investigation has been carried out with various parts of plant shoots and various pigment concentrations. It is demonstrated that the rate of the metabolic process responsible for inclusion of C^{14} in chlorophyll is different in various parts of the shoot and decreases upon moving from the upper part of the shoot to the lower part. The time required for establishment of maximal specific activity increases in a similar manner. The gradient is especially noticeable in the case of chlorophyll b. The radioactivity of this pigment increases at a slower rate, and its maximum is attained at a later period than in chlorophyll a. This is taken to mean that the path of carbon to chlorophyll b is longer than that traversed before inclusion in chlorophyll a. The increase of the radioactivity of chlorophyll several days after the plant was removed from the C^{14}-containing medium indicates that chlorophyll is not formed as a result of photosynthesis but as a result of the usual metabolism of assimilatory products. Only several tenths of a percent of the C^{14} incorporated was found in chlorophyll, and its specific activity during the one and a half month duration of the experiments remained below that of carbon in the tissue as a whole. Chlorophyll is therefore more conservative than many other substances and is involved in the general metabolism at a smaller rate.

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ON VARIATION OF THE CHLOROPLAST SIZE AND PIGMENT CONCENTRATION DURING PROLONGED DARKENING FOLLOWED BY ILLUMINATION

T. N. Godnev, A. V. Leshina, and L. A. Khodorenko

V. I. Lenin Belorussian State University, Minsk
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In our previous studies [1,2] we pointed out the existence of a wide literature concerning the effect of illumination on the pigment system of plants. Since that time many studies have appeared on this problem. In these studies two tendencies are noted: Followers of Willstätter, Seybold, and his school [3] maintain that the chlorophyll content within the plant is almost constant and changes slowly, while Bukach and collaborators indicate a possibility of marked variations in the content of pigment even during a single day. In a thorough study Bauer [4] has shown little likelihood of such marked variations when the amount of pigment is calculated not on its dry or raw weight but by unit area, when samples are taken at different locations of the same leaf.

In this study we shall deal with only one of a large number of problems: the variation in plastids and their pigment content under conditions of prolonged complete darkness.

From Saks's time almost up to the present it was commonly accepted that green plants can be divided into two groups as regards reaction to an illumination regimen. To the first group belong the plants capable of synthesizing chlorophyll not only in light but also in complete darkness, even though to a considerably lesser degree. Among these are many algae and other thallophyta; also gymnospermae in the sprouting stage while they preserve contact with the endosperm. To the second group belong angiospermal plants in which chlorophyll formation could be observed only in light by the formerly existing methods, while in darkness these plants were evidently devoid of green coloration, and optical methods disclosed only the presence of carotenoids and protochlorophyll. To our knowledge, Seybold and Egle [5] were the first to discover the capacity for synthesizing chlorophyll in some grains, kidney beans, and garden peppergrass, but not in initially etiolated plants: only in those which first acquired on green coloration and were subsequently placed in darkness again for a prolonged period. The character of such chlorophyll synthesis under conditions of darkness was quite unique. The plants, after being placed in darkness, displayed a noticeable decrease in the quantity of chlorophyll for a prolonged time and then began to accumulate it, after which a new decrease followed. These fluctuations in chlorophyll content were quire indefinite, and therefore the studies did not attract the attention which they undoubtedly deserved. However, even the authors themselves did not offer an explanation of the fluctuations they observed in the synthesis and decomposition of chlorophyll. Later on, a very small extent of chlorophyll synthesis was observed by Goodwin and Owens [6] in totally etiolated oat plants. The quantity of chlorophyll, judging by the cited spectrophotometric curves, synthesized per g of raw material, amounted to only several hundredths of a microgram. Approximately within the same magnitude was the chlorophyll quantity found by Robelen [7] in 1956 in leaves of arabidoxis grown in complete darkness.

In our joint study with N. K. Akulovich small quantities of chlorophyll were found in etiolated leaves of canna, one portion of which was fully shaded and the other illuminated.

In our study with Shlyk and Rotfarb [8] we found an inclusion of C^{14} in the chlorophyll molecule by some water plants and land plants when the former were placed in water which contained the anion $C^{14}O_3$ and the land plants in an atmosphere which contained $C^{14}O_2$. In these cases either all or the largest portion of active carbon atoms was concentrated in the phytolic portion of the molecule. We consider the following hypothesis probable: The well-developed enzymatic system of lower plants capable of accomplishing chlorophyll synthesis in complete absence of light, and most probably, of converting protochlorophyll into chlorophyll a, or protochlorophyllid into protochlorophyllid a, gradually degenerated in the process of evolution. However this degeneration in some angiospermae was not complete and was preserved in a so-called rudimentary stage, so that such plants preserve to a certain degree the capacity to reduce protochlorophyll to chlorophyll. By all the evidence, this capacity was somewhat increased when the

TABLE 1. Changes in Quantity and Volume of Plastids, and in Chlorophyll Content, Affected by Darkening and Subsequent Illumination of Spearleafed Pond Weed

Date	Experimental variant	Plastid quantity	Plastid volume, μ^3	Chlorophylls a + b, mg of dry weight	$\frac{a}{b}$
	Darkened for 12 days				
7.VII	control	7	26.0	1.000	2.7
	after darkening	6	18.8	0.155	1.0
11.VII	control	6	26.0	0.835	1.6
	illuminated 4 days	7	15.8	0.269	1.4
15.VII	control	7	26.5	0.908	2.0
	illuminated 8 days	6	21.8	0.857	2.2
19.VII	control	7	26.0	1.071	1.9
	illuminated 12 days	7	25.5	0.933	2.0
	Darkened for 16 days				
11.VII	control	6	26.0	0.835	1.6
	after darkening	6	9.5	0.252	1.0
15.VII	control	7	26.5	0.903	2.0
	illuminated 4 days	7	15.5	0.403	2.3
19.VII	control	7	26.0	1.071	1.9
	illuminated 8 days	7	16.0	0.949	2.0
23.VII	control	7	26.5	1.248	2.5
	illuminated 12 days	7	22.0	1.484	2.2
	Darkened for 20 days				
15.VII	control	7	26.5	0.908	2.0
	after darkening	4	9.4	0.189	1.7
19.VII	control	7	26.0	1.071	1.9
	illuminated 4 days	5	11.6	0.313	2.1
23.VII	control	7	26.5	1.248	2.5
	illuminated 8 days	6	14.0	0.521	2.0

plant was illuminated before being kept in darkness and accumulated a certain reserve of energy material stimulating the activity of enzymatic systems mentioned earlier. This point of view was confirmed in recent experiments by Virgin [9] on stimulating protochlorophyll accumulation as well as chlorophyll formation when etiolated sprouts are illuminated for a short period of time. However, most angiospermal plants completely lost the enzymes reducing protochlorophyll. Thus, though some definite evidence has been accumulated concerning changes in pigment content in the absence of light, considerably less is known about the character of changes observed in the plastid apparatus in absence of light.

Wolken and Schwertz [10] observed that total decomposition of euglenia chloroplasts occurs on prolonged exposure to darkness. Single granules of these chloroplasts lost their lamellar structure in darkness and became almost homogeneous, but on illumination their normal structure returned.

In the present study we shall investigate the problem of tracing changes in the chlorophyll content as well as in dimensions and quantities of plastids in some water plants characterized by shade endurance (spearleafed pond weed and eel grass). This, we thought, would shed light on the question as to whether the accumulation and decomposition of chlorophyll occurs parallely with the change in the general mass of plastids, or is perhaps due to the range of the pigment in the elements of the plastid itself and in the granules.

Similar observations were also conducted on typical heliophilic plants – corn and potato. It was also of interest to determine for how long a time the ability is preserved to regenerate plastids and accumulate chlorophyll after various periods of remaining in darkness by different types of plants in relation to the light regimen.

EXPERIMENTS WITH WATER PLANTS
(pond weed and eel grass)

On a shallow bank of Lake Naroch, about 30 cm deep, during June-July bushy growths of spearleafed pond weed were collected and shaded in boxes impervious to light for 4, 8, 12, 16, and 20 days. Eel grass plants were placed in a dark chamber for 5, 10, 15, and 20 days. As control, plants were left permanently in light.

In order to establish the reaction to darkness of the plastid apparatus and the pigments contained in them as related to their ability to regenerate to normal dimensions and to form pigments, the plants were placed in light after a definite period in darkness.

Every 4 days samples were taken from pond weed leaves by a drill 7.5 mm in diameter. Discs obtained by a previously described method [11] were counted and measurements were taken of the size of the plastids, after which chlorophylls a and b were determined in these samples.

For determining the volume of plastids and the general quantity of chlorophyll (a + b) in eel grass, a small piece of the leaf was taken.

TABLE 2. Effect of Darkening and Subsequent Illumination on the Volume of Plastids and Chlorophyll Content in Eel Grass

Date	Experimental variant	Plastid volume, μ^3	Chlorophyll, mg/g of raw material
	Darkening for 5 days		
4.II	control	24.3	0.922
	after darkening	8.0	0.629
9.II	control	23.3	0.517
	illumination for 5 days	21.8	0.568
11.II	control	19.2	0.710
	illumination for 7 days	18.0	0.808
	Darkening for 10 days		
9.II	control	23.3	0.517
	after darkening	6.1	0.242
14.II	control	26.7	0.958
	illumination for 5 days	16.5	0.476
16.II	control	25.6	0.710
	illumination for 7 days	22.5	0.790
	Darkening for 15 days		
14.II	control	26.7	0.958
	after darkening	7.5	0.490
19.II	control	27.5	0.850
	illumination for 5 days	18.3	0.588
	Darkening for 20 days		
19.II	control	27.5	0.850
	after darkening	9.0	0.475
24.II	control	23.9	0.978
	illumination for 5 days	14.4	0.697
26.II	control	25.4	0.970
	illumination for 7 days	15.9	0.724
28.II	control	23.3	0.764
	illumination for 9 days	15.3	0.697

TABLE 3. Effect of Shading and Subsequent Illumination on the Size of Plastids and Chlorophyll Accumulation in Potatoes

Date	Experimental variant	Plastid volume, μ^3	Chlorophylls a+b, mg of dry weight	$\frac{a}{b}$
	Shading for 2 days			
17.VII	control	20.5	4.276	3.5
	after shading	11.8	4.342	3.9
19.VII	control	18.8	3.051	3.2
	illumination 2 days	15.7	3.743	3.8
	Shading for 4 days			
19.VII	control	18.8	3.051	3.2
	after shading	4.9	0.347	2.1
21.VII	control	12.5	5.323	3.1
	illumination 2 days	6.3	1.766	4.5
23.VII	control	17.0	3.669	3.3
	illumination 4 days	17.0	—	—
	Shading for 6 days			
21.VII	control	12.5	5.323	3.1
	after shading	1.4	0.570	1.3
23.VII	control	17.0	3.669	3.3
	illumination 2 days	9.0	1.478	3.5
25.VII	control	27.2	3.739	4.2
	illumination 4 days	11.7	2.850	3.3
	Shading for 8 days			
23.VII	control	17.0	3.669	3.3
	after shading	1.4	0.488	2.4
25.VII	control	27.2	3.739	4.2
	illumination 2 days	2.2	0.274	1.6

TABLE 4. Effect of Shading and Subsequent Illumination on the Plastid Volume of Corn

Date	Experimental variant	Plastid volume, μ^3
	Shading for 5 days	
31.I	control	22.6
	after shading	14.8
5.II	control	30.8
	illumination for 5 days	15.9
7.II	control	30.8
	illumination for 7 days	23.3
	Shading for 10 days	
5.II	control	30.8
	after shading	5.3
10.II	control	24.1
	illumination for 5 days	13.3
	Shading for 15 days	
10.II	control	24.1
	after shading	0.85

The results of the experiment on studying the course of changes in plastid dimensions and chlorophyll accumulation in pond weed are presented in Table 1.

It is seen from Table 1 that darkening for 12 days decreases the plastid volume from 26.0 μ^3 down to 18.8 μ^3. After illumination for 12 days, restoration of plastid volume was observed. Similarly, this occured in a variant when the darkening lasted 16 days. Even a 20-day darkening does not destroy the plastids; it only decreases its volume to $\frac{1}{3}$.

In determining the quantities of both chlorophyll components (a and b) it was found that the control retains a ratio, characteristic of water plants, of chlorophyll a to chlorophyll b of 1.8 : 2. It is known that chlorophyll a is less stable than chlorophyll b.

In our experiments, as the dark period is lengthened the ratio of chlorophyll a to chlorophyll b falls to 1.0: 1.4. However, subsequent illumination restores it to the initial magnitude. The total quantity of chlorophyll decreased in proportion to the darkening and increased after the darkening shield was removed.

It is interesting to note that the quantity of plastids on darkening even as long as 16 days is not subjected to change. This means that the process of chlorophyll decomposition occurs within the plastid apparatus, while it remains intact and preserves its capacity of restoring normal vitality and even chlorophyll formation.

Similar results were obtained in experiments with eel grass, when exposure to 5-20 days of darkness was tested (Table 2). In this case also the plastids, even though they decreased in size, did not preserve their vitality after a normal light regimen was restored; their volume gradually increased. On prolonged darkening (10-12 days), a decrease in the quantity of chlorophyll to 1/3 was observed in eel grass, but on subsequent illumination the chlorophyll content returned almost to the control level.

Thus the investigations conducted on water plants furnish a basis for assuming that the disrupted chlorophyll-protein-lipoid structure after a relatively prolonged darkening is again capable of synthesis when normal light conditions are restored.

EXPERIMENTS WITH POTATOES AND CORN

Experiments were also conducted on the effect of darkening in relation to pigment content and the size of plastids in plants most sensitive to changes in the light regimen. The experiments with potatoes were conducted in field environments on mature plants on the eve of budding. Young (5-day-old) corn sprouts, cultivated in transplanting boxes, were used for shading. Shading for 2 and 8 days was tested on potatoes; for 5 and 15 days on corn.

The data of the effects of various periods of shading and subsequent illumination on the size of plastids and accumulation of chlorophyll in potatoes are presented on Table 3.

It is seen from Table 3 that 2 days of shading of potato plants caused almost no changes. The experimental variant coincided with the control in chlorophyll content. Dimensions of plastids decreased (control, 20.589 μ^3; the experimental variant, 11.807 μ^3), but on subsequent illumination for 2 days they reached the dimensions of the control again.

Shading for 4 and 6 days decreased the amount of chlorophyll, in comparison with the control, 9.5 times. Considerable changes occured in the plastid apparatus. On shading for 4 days the plastids were decreased to $\frac{1}{4}$, and on shading for 6 days, to $\frac{1}{9}$ compared to the control. It should also be noted that in both these variants an angularity and an indistinct form of the plas-

tids was observed, which is evidently related to the beginning of decomposition. But on subsequent illumination the size of plastids and the amount of chlorophyll are gradually restored. The angularity of plastids disappears, they take on the former form. Thus, on shading for 4 days and illumination for 4 days the size of the plastids returns to that of the control.

It is true that in plants kept in darkness for a period of 6 days, the increase in amount of chlorophyll and size of the plastids proceeds at a much slower rate, but the possibility of their restoration on subsequent illumination is evident.

On shading for 8 days, dying off of the leaves in the lower and middle ranges was observed. The quantity of chlorophyll decreases from 3.669 mg/g of dry substance (control) down to 0.488 mg/g of dry substance, and the plastid dimensions from 17.025 μ^3 (control) down to 1.385 μ^3, while there are almost no clearly defined plastids. Subsequently illumination produces no effect. The quantity of chlorophyll continues to decrease down to 0.274 mg/g of dry substance.

Shading of corn sprouts for 5 days (Table 4) caused yellowing of leaves and a decrease in plastids from 22.5 (control) down to 14.8 μ^3. Subsequent illumination somewhat increases the size of plastids, but this proceeds at a much slower rate than in potatoes. Even an illumination of 7 days does not restore the volume of plastids to that of the control (control, 30.83 μ^3; the experimental variant, 23.30 μ^3). The plastid apparatus of corn sprouts proved to be more conservative to restoration of its previous dimensions on subsequent illumination, but more resistant to shading. The plastid vitality was preserved even on a shading of 10 days. And only a 15-day-long shading led to a sharp decrease in the volume of plastids (24.15 μ^3, control; 0.852 μ^3, the experimental variant) and to the destruction of the plant.

Thus, in short-period shadings of 2 to 6 days in potatoes and of 5 to 10 days in corn sprouts, the temporarily disturbed activity of the photosynthesizing apparatus is capable, after a definite exposure to light, of restoring itself anew. A more prolonged shading (8 days in potatoes, 15 in corn) leads to irreversible changes in the plastid apparatus.

SUMMARY

The effect of prolonged complete darkening with subsequent illumination of varying duration on the plastid size and chlorophyll content in leaves of shade and light plants was studied.

During the first 6-8 days in the dark the plastid volume of shade plants decreased concurrently with decrease of the amount of chlorophyll. After 12-16 days the plastid volume and chlorophyll content sharply dropped. However, illumination after 12-day darkening almost completely restored the plastid size and chlorophyll content; the restoration was incomplete when illumination followed a 16-day dark period.

The number of plastids in the cell did not change. After 20 days of darkness the plastids are partially destroyed, and they lose their ability to regenerate. Chlorophyll a degrades much more rapidly than does chlorophyll b. The a/b ratio decreases by two times after 12-16 days darkness and is restored to the initial value after illumination.

In sun (heliophilic) plants (potato) the plastid volume and pigment content rapidly decrease during the first days of darkness, and the plastids soon lose their ability to regenerate.

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SEASONAL CHANGES IN SPRUCE CHLOROPLASTS

P. A. Genkel' and E. I. Barskaya

K. A. Timiryazev Institute of Plant Physiology, Academy of Sciences of the USSR, Moscow

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The status of the plastid apparatus of evergreen leaves, including conifer needles, is of considerable interest in connection with the general topic of dormancy and frost resistance.

As is well known, dormant plant cells undergo profound physiological and biochemical changes, leading to a marked reduction in physiological activity [1-7].

Reduction of photosynthesis to minimal levels, or even its cessation, which is observed in the winter time [8-14], can also be explained in terms of profound changes in the plastid apparatus. A number of studies of seasonal changes in plastids of evergreen leaves, and also of the cortex of certain woody species, are known. This has proven to be a complex subject, and the information is quite contradictory, so that a clear representation of the character of these changes has not yet been made.

Kraus [15] and Senn [16] observed a shift in position of chloroplasts in the leaves of certain overwintering herbaceous plants. Working with fixed material, Ryazantsev [17] established that in conifer needles and the leaves of certain other evergreens the chloroplasts become closely packed in the cell during the winter, while maintaining their individual integrity. Mikul'skaya [18] found the same thing in box leaves. Recently Holzer [19]* carried out in vivo observations of seasonal changes in spruce and pine chloroplasts in the high alpine forests of Austria and found that in the winter there is an aggregation of chloroplasts without loss of their identity.

Other workers have found more complex phenomena involving aggregation into a homogeneous mass in which the plastids lose their individuality entirely; this type of phenomenon has been designated as agglutination [20-27]. Some workers have observed chloroplast disintegration and conversion into "diffuse chlorophyll" [28, 29], with diffusion into the cytoplasm and the cell walls [30].

Recent electron microscope studies of chloroplasts in overwintering Bellis perennis plants [31-33] have convincingly shown that during transition of cells to the dormant state there is a profound internal reorganization of the chloroplasts which leads to alteration of their physiological condition and cessation of photosynthesis. The structural changes are not associated with disintegration and agglutination but are, on the contrary, such as to render the chloroplasts more compact. Examination of cells under both electron and light microscopes has shown the intact chloroplasts to be scattered through the cell.

As noted by some of the previously mentioned workers, the nucleus undoubtedly plays a role in changes of the plastid apparatus. A relationship between the nucleus and amyloplasts of starch-forming cells of potato has been demonstrated [34].

Recently the Japanese investigator Joshida [35] showed experimentally a correlative relationship between the nucleus and chloroplast activity. By plasmolyzing Elodea leaves in 0.2M $CaCl_2$, he obtained cells in which the protoplast had divided into two parts, one with the nucleus and the other without. Over a period of several days a continous starch accumulation in swelling chloroplasts was observed in the anucleate part and a disappearance of starch from shrinking chloroplasts in the nucleate part. It was thus established that plasmolysis does not affect the plastids directly, but only as a consequence of inducing a paranecrotic condition in the nucleus. Moreover, it was shown that with addition of small amounts of inhibitors to the plasmolyzing solution (NaF, NaN_3, DNP, KCN) starch disappearance and chloroplast shrinkage in the nucleate part were more rapid than in the anucleate part, in which a certain decrease in chloroplast size and also, in two out of four cases, a disappearance of starch was observed. From these data Joshida concludes that the nucleus evidently affects the chloroplasts enzymatically.

PURPOSE AND METHODS

This investigation was undertaken to determine the extent of changes in spruce chloroplasts during the winter, their overwintering condition, and the mode of restoration of the plastid apparatus to an active state during the plant's transition from dormancy to active growth. The study was carried out in the temperate zone of the USSR (Moscow).

*Unfortunately we became aware of this study after we had completed our experiments.

Chloroplasts of spruce (Picea excelsa Link.) were studied microscopically in 1959-1960. Since dormancy in one- to two-year-old shoots is less profound than in old shoots, it was of interest to trace chloroplast changes in needles of various ages; to this end a comparative analysis of needles from shoots of different ages on a single tree was made.

We considered it extremely important to injure the cells as little as possible in preparation of the material, since preliminary results had shown that chloroplast destruction sets in rapidly in necrotic cells, and changes associated with death can easily be mistaken for those peculiar to the overwintering condition. In vivo observations of sections were therefore made without preliminary staining, chiefly in neutral medium, vaseline oil, which does not cause injury to chloroplasts as do glycerin, water, or other media. In addition to cross sections, lengths of whole, unsectioned needles with the cuticle partially removed and longitudinal halves were studied. This type of observation was possible after preliminary infiltration of the needle with vaseline oil [36], the preparation being examined with strong illumination under an immersion lens.

Although living cells differ from dead ones by the presence in the latter of a clearly visible nucleus, we used the plasmolytic method to distinguish the two kinds. Sections were first quickly examined in water, and then plasmolyzed in 1M NaCl or 1-2M sucrose. This was necessary in order to obtain the most reliable data on the chloroplast condition of living cells.

Starch content of chloroplasts was determined histochemically with Lugol's solution as a measure of photosynthetic activity at different times of the year.

In vivo observations were supplemented by study of material treated with Riegel's solution, a good plastid fixative. Permanent preparations stained with Heidenhain's iron hematoxylin and counterstained with light green were made by the method commonly employed. Longitudinal and transverse sections, which were 10-14μ thick, were examined and drawn with a $40\times$ eyepiece and a $90\times$ immersion objective.

RESULTS

Winter samples from needles of all ages exhibited an exceptionally marked aggregation of chloroplasts around the nucleus, which was masked as a result. In transverse sections, groups of clumped chloroplasts are visible near one of the cell walls, the remaining part of the cell being free of chloroplasts and colorless (Fig. I, 1, 3). In longitudinal halves or whole mounts, the same thing is seen, but often the mass of chloroplasts and nucleus occupies a position in the center of the cell (Fig. I, 1c).

These configurations, typical of the winter condition, are clearly evident in both living and fixed material.

It should be noted that in permanent preparations the chloroplast aggregates are often looser than in living material (Fig. I, 3a, b); apparently there is a certain dispersion of chloroplasts at the time of fixation. This can serve as proof that they do not lose their identity in the winter.

The growing chloroplast mass is not oriented toward the poles of the cell, as is the case in certain other plants. In all cells examined, the center of aggregation was always the nucleus.

As is well known, a close contact between nucleus and cytoplasm is established during dormancy. Evidently chloroplast aggregation leads to the establishment of close contact between the nucleus and plastids as well, being the visible manifestation of complex physiological processes developing in the resting cell.

With careful study of living material under high magnification, individual dark green, lens-shaped (seen edgewise) chloroplasts which are closely appressed to one another can be distinguished. The central part of the mass has a green background, which evidently represents a mass of chloroplasts below the focal plane. Along the periphery of the dark green mass, rounded (seen from above) light green chloroplasts are visible (Fig, I, 1b, c). In occasional cells chloroplast contours cannot be made out, only a general diffuse mass being distinguishable; plasmolysis ordinarily does not occur in such cells, indicating that they are injured. It was also noted that cell injury rapidly induces chloroplast destruction.

In a few cases, cells are encountered with a chloroplast distribution characteristic of summer (evenly dispersed), but such cells are not typical of the winter condition.

Upon exposure of spruce branches to room temperature for three to five days, many cells exhibit disaggregation of the chloroplast mass, with dispersion of the plastids through the cytoplasm (Fig. I, 2). This occurs first of all in one-year-old needles and subsequently in older needles.

A distinctive feature of the one-year-old needle is the presence of colorless cell inclusions of various sizes and shapes, ranging from rounded to angular, with a marked light-refracting capacity; these inclusions are considerably larger in needles many years old. Treatment of sections with Scharlach's and Lugol's solutions yielded no clue as to the nature of these inclusions; they were neither lipoidal bodies nor starch grains. They were not leucoplasts, since they gave a negative reaction for protein. We assumed, moreover, that as possible precursors of chloroplasts, leucoplasts should have occurred in greater amounts in the young needle and in lesser amounts, or not at all, in an old needle. In fact, however, the inclusions always occurred in greater amounts in old needles. A solution was found in the statement of Dzhaparidze [37] to the effect that old leaves of evergreen plants often contain so-called assimilation secretions and mesophyllic secretions, while young ones contain little or none. It is noted that "young, vigorously

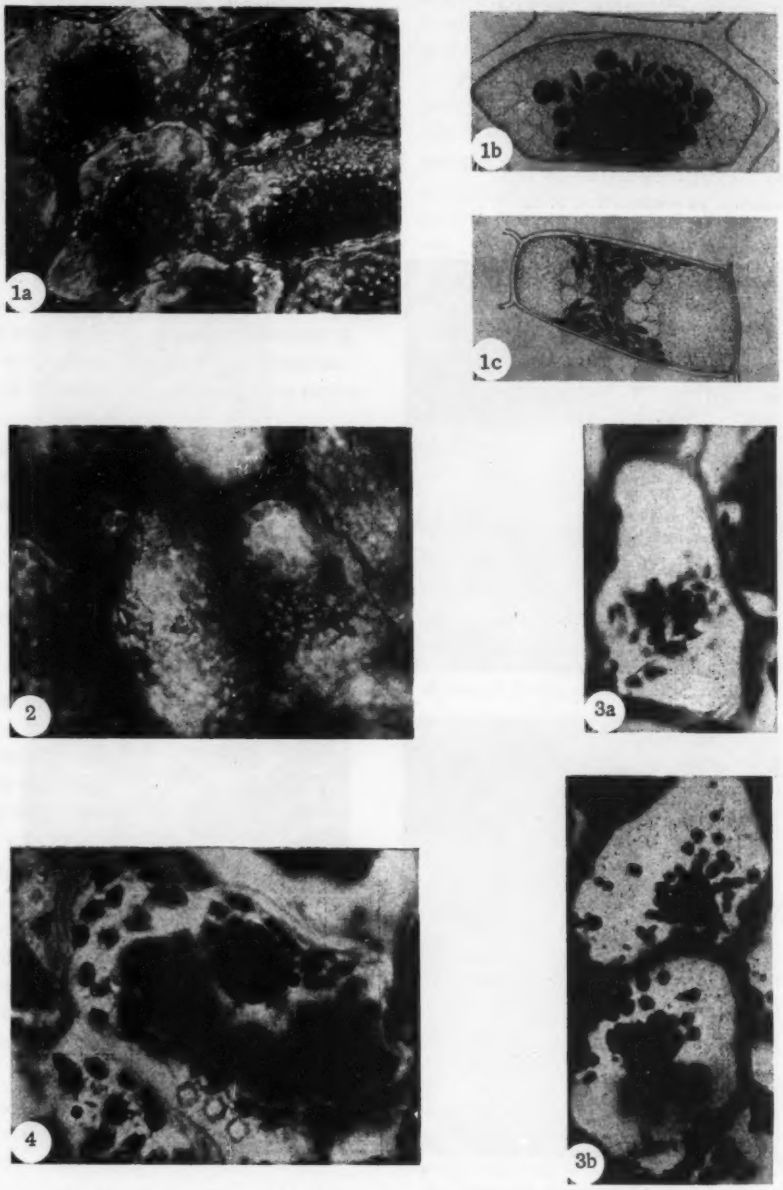

Fig. I. Seasonal changes in spruce chloroplasts. 1a) Winter; clumped chloroplasts in living cells; transverse section in vaseline oil (photomicrograph); 1b) the same as 1a but only one cell shown; 1c) winter; cells of part of a needle with cuticle removed, infiltrated with vaseline oil (drawing); 2) winter; even distribution of chloroplasts after five days in the laboratory (transverse section, photomicrograph); 3a) winter; clumped chloroplasts in fixed cells of a one-year-old needle (permanent preparation, hematoxylin, photomicrograph); 3b) the same, but in cells of a four-year-old needle; 4) spring, even distribution of chloroplasts (permanent preparation, hematoxylin, photomicrograph).

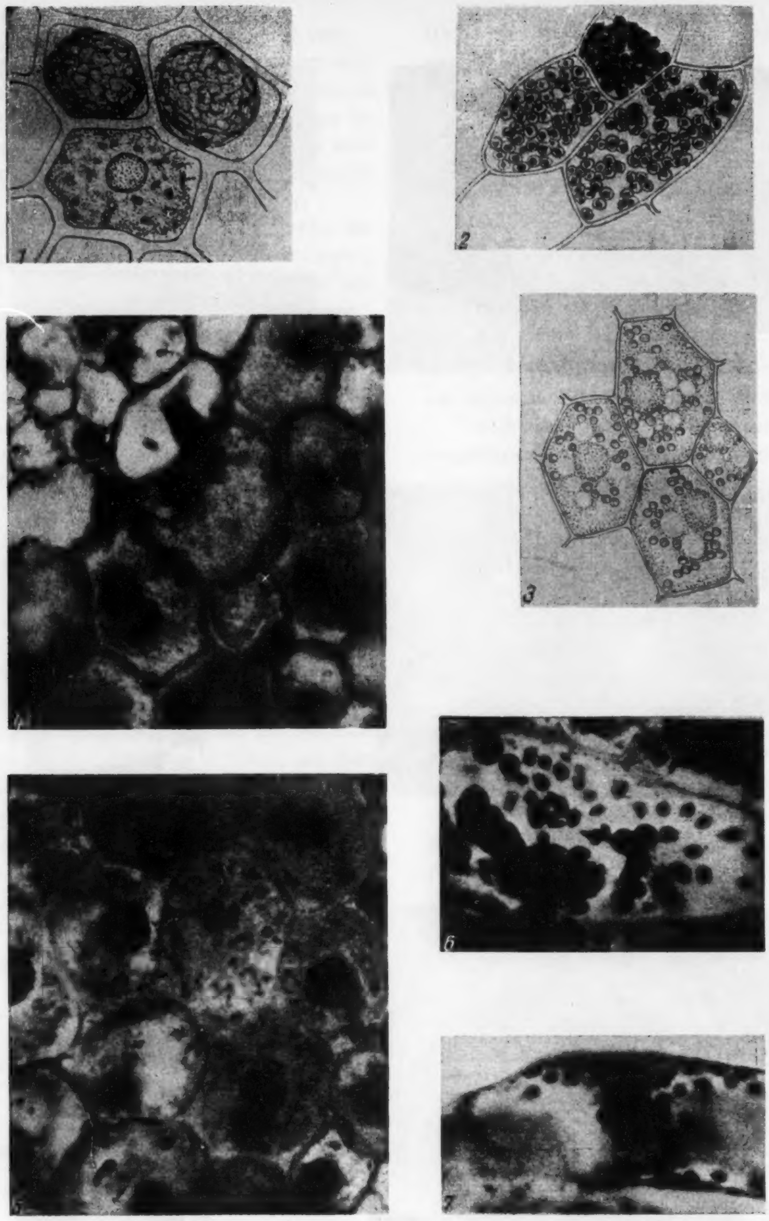

Fig.II. Seasonal changes in spruce chloroplasts. 1) May; cells of an actively metabolizing needle in plasmolyzing solution after cooling; plasmolysis in living cells, nucleus not visible, hyaline starch grains in chloroplasts; no plasmolysis in dead cell, nucleus visible, chloroplasts disintegrated (drawing); 2) May; cells of an old needle, starch in chloroplasts (Lugol's solution, drawing); 3) May; cells of a young needle of new growth, small chloroplasts visible, also nucleus and vacuoles (drawing); 4) May; binucleate condition of a growing needle on new growth; plastids not visible (permanent preparation, hematoxylin, photomicrograph); 5) the same, but forming plastids visible (permanent preparation, hematoxylin); 6) end of October 1959; beginning of chloroplast aggregation in one-year-old needle (permanent preparation, hematoxylin photomicrograph); 7) the same, but a four-year-old needle.

growing organs seldom exhibit such secretions. In deciduous leaves, on the other hand, and also in evergreen leaves under unfavorable conditions, they occur in the greatest amounts." In the winter, the most unfavorable period in the life of the plant, we find these inclusions in the greatest numbers, there being considerably more in an old needle than in a young one.

Dzhaparidze states that the physiological significance of these substances has not been determined. Some workers regard them as secretory products similar to starch in function, while others consider them as waste materials. At present there is some doubt as to the view that, once formed, these secretions are metabolically inert. It may be noted that the inclusions in spruce needles do not remain unaltered, but disappear during the period of transition from dormancy to active growth.

In the middle of March, 1959, it was observed that in one-year-old needles the chloroplasts had already separated from the mass and were fairly evenly distributed in the cell; in a few cells, chlorophyll was in a diffuse condition, indicating that cell injury had occurred. In four-year-old needles from the same shoots, chloroplasts were still strongly aggregated. After these shoots had been kept at room temperature for five days, the condition of chloroplasts in one-year-old needles was unchanged, while that of chloroplast aggregation was no longer visible, and the chloroplasts were evenly dispersed throught the cell. By the end of March, the process of disaggregation had progressed further (Fig. I, 4); only in the four-year-old needle was a transitional condition still evident.

In 1960, spring was considerably delayed, and the winter configuration of chloroplasts in needles of all ages was maintained up till the middle of April; only in isolated cells of a one-year-old needle had they separated and assumed the distribution pattern typical of summer. As the disaggregation progressed, there was a decrease and disappearance of the colorless inclusions.

It should be noted that in winter and early spring samples, tests for starch were negative, indicating that the plastids were not functioning and starch was not being formed.

At the end of April, 1960, the summer configuration was observed in needles of all ages. At this time the terminal buds had barely swelled but had not opened, and the needles had begun to metabolize actively. Tests with Lugol's solution showed that each chloroplast contained a more or less prominent starch grain (Fig. II, 2). In all cells the peripheral portion of the chloroplasts was clearly visible. It was especially striking in permanent preparations stained with hematoxylin, where the starch was light in color and hyaline, and the stroma, the peripheral part, was black.

It was of interest to see whether it would be possible to induce the winter distribution of chloroplasts by subjecting them to low temperatures. In other words, it was important to determine whether temperature is the only factor determining the unique behavior of chloroplasts in the winter, or whether aggregation is a visible manifestation of complex intracellular processes occurring in the winter which have evolved in response to a complex of environmental factors, such as temperature, light intensity, day length, etc., with a periodicity of action.

Freshly cut spruce branches were exposed by stages to freezing temperatures in a phytotron.

The data of Table 1 show that after the needle was cooled at −5 and −10° for a day, a considerable number of cells were still living, although injured cells were already being encountered. Here it is interesting to note that in living cells the protoplasmic viscosity is enormously increased. Plasmolysis took place after 25-30 min, while in uncooled needles it occurred after 5-8 min. These data are in agreement with those of P.A. Genkel' et al. [38-42] to the effect that protoplasmic viscosity is a measure of cell viability, physiological activity being low with high viscosities and vice versa.

There were no apparent changes in chloroplast condition of living cells at low temperatures, and the summer configuration was preserved. After a further, also prolonged cooling at −20°, death occurred, as manifested in untreated sections by the appearance of a clearly visible nucleus and a coarsely granular protoplasm, and also by failure of plasmolysis to occur. Even prior to death, no aggregation of chloroplasts around the nucleus was observed. In the dead cells, chloroplasts disintegrating and fusing into a conglomerate mass could be seen (Fig. II, 1).

In a second experiment branches of the same age were subjected to more prolonged freezing – two to four days at −3, −5, and −10° (Table 2).

From the data presented it is clear that prolonged exposure to these temperatures leads to increased protoplasmic viscosity and cell death, but chloroplast distribution remains unchanged. With cell death, the chloroplasts disintegrate. This experiment has thus confirmed the common assumption that cells which have emerged from dormancy are much less frost resistant and succumb to temperatures which are without effect on dormant cells.

Furthermore, low temperatures have no effect on chloroplast distribution in those cells which do survive. This confirms our hypothesis that the massing of chloroplasts around the nucleus, which is typical of winter, is not a consequence of low temperature alone. As already pointed out many times [1-7], in the resting cell there are complex physiological, biochemical, and morphological changes which predispose to high resistance.

TABLE 1. The Effect of Successive Coolings on Condition of Chloroplasts in Spruce Needle Cells (End of April, 1960)

cooling temperature, °C	duration of expt., hr	condition	
		of cells (according to plasmolysis behavior)	of chloroplasts in cells
−3	3	living	even distribution
−5	20	the same	the same
−10	6	living; a few cells injured	even distribution; chloroplast destruction beginning in dead cells
−20	17	dead	chloroplast destruction

TABLE 2. The Effect of Successive Coolings on Condition of Chloroplasts in Spruce Needle Cells (End of April, 1960)

cooling temperature, °C	duration of expt., hr	cell condition	chloroplast condition
− 3	48	living; some dead (more in one -year-old needles than in those several years old); increase in protoplasmic viscosity	even distribution; chloroplast destruction in dead cells
	96	few living; many dead	the same
− 5	42	living; some dead; increase in protoplasmic viscosity	"
	92	living; more than half dead	"
−10	48	considerable number dead; paranecrosis in a few cells (abnormal plasmolysis)	chloroplast destruction

The so-called embryogenic processes, which lead to cell activation after emergence from dormancy, are also in motion.

The chloroplast aggregation which occurs in winter under natural conditions cannot be induced experimentally by low temperatures alone, as our experiments have shown. This indicates that seasonal changes in chloroplasts, which have evolved in response to a complex of environmental factors which undergo periodic alterations during the year, are one of the manifestations of profound intracellular changes associated with transition to dormancy. The assumption follows that contact of chloroplasts with the nucleus in the winter time may represent a protective reaction to low temperatures of the extremely susceptible plastids. Moreover, such contact may activate the chloroplasts, preparing them for intensive activity after emergence from dormancy.

In the second half of May there appear at the ends of shoots small, 2-3 cm growths, or closely grouped fascicles of young, light green needles, which are very soft, without a developed mechanical tissue.

Analysis of the cells of these young needles revealed substantial differences from the cells of older needles. The most obvious feature of living sections is the small number of light green plastids in the cell and their small size, which is often half that of chloroplasts of an old needle. Chloroplasts are concentrated mainly around the nucleus and around small vacuoles, and very few are found in the cytoplasm between vacuoles (Fig. II, 3). In these cells, the nuclei are clearly demarcated and apparently play an important role in formation of the plastid apparatus. Young plastids in needles of the new growth photosynthesize, as shown by a positive reaction for starch. Study of permanent preparations stained with hematoxylin showed that cells of a young needle whitish at the base do not contain typical chloroplasts, but do contain a few characteristic bodies which are evidently plastid precursors (Fig. II, 4, 5). Here it is interesting to note, however, that no mitoses were found in growing tissues of a yound needle stained with hematoxylin; only compact, interkinetic nuclei and loosely organized nuclei in a condition reminiscent of the prophase

stage are encountered. In addition, binucleate cells are seen very often (Fig. II, 4, 5).

It is well known that in many cases a non-mitotic type of diversion takes place in growing tissues. In such tissues the number of mitoses is insignificant, or they are completely absent, and in many cells a multinucleate condition (two or more nuclei) occurs, indicating that non-mitotic divisions of the nuclei are taking place [43-45].

Recent studies have shown, in contradiction of previously held views, that amitosis is not a pathological phenomenon foreshadowing suppression of tissue activity. It was shown that amitosis is widespread in both the plant and animal world and that it represents a biologically efficient method of cell and nuclear division, even being in some cases the preferred form of division for ensuring tissue growth and differentiation. It is emphasized, however, that mitosis in not equivalent to amitosis, since the latter is found only in cells and tissues not essential to reproduction. In this connection, there is still no unanimity of opinion as to which of the two possible types of cell division ensures the most rapid tissue growth [46, 47]. Evidently the amitotic type is the main type occurring in a young needle.

Let us return to the material analyzed. In summer and fall chloroplasts are evenly distributed in the cell in the manner observed at the end of spring. Accumulation of the secretory granules begins in the latter half of the summer, when colorless grains are seen in a one-year-old needle and larger colorless inclusions, of rounded or sometimes angular form, are seen in older needles. At the end of October and beginning of November the first signs of a change in the plastid apparatus appear. In many cells an incipient aggregation of plastids around the nucleus can be made out on a background of evenly distributed chloroplasts. Here the relation between seasonal changes in chloroplasts and the age of the needle should be emphasized once again. In spruce, aggregation begins in older needles and then spreads upwards along the shoot to the one-year-old needles (Fig. II, 6, 7).

SUMMARY

1. Spruce chloroplasts undergo seasonal changes which are manifested in a change in their distribution in the cell. While in an actively metabolizing plant they are evenly distributed in a continous protoplasmic layer, in dormant plants they are aggregated around the nucleus.

2. In dormant spruce needles a close contact between nucleus and chloroplasts is established which entails a change in their physiological status. This contact represents one of the manifestations of complex physiological processes taking place in the dormant cell.

3. Seasonal changes in chloroplasts evolved in conditions of alternating warm and cold periods under the stimulus of a complex of environmental factors (day length, light intensity, temperature, etc.).

It has proven impossible to induce the winter pattern of chloroplast distribution in actively metabolizing cells with a summer distribution by the application of low temperatures alone.

4. Aggregated chloroplasts in dormant cells do not photosynthesize (absence of starch in plastids); with emergance of the plant from dormancy, chloroplasts once more become disposed throughout the surface layer of protoplasm and resume active photosynthesis (starch in plastids).

5. Needle age plays a definite role in seasonal changes of the plastid apparatus. In an old needle, the winter configuration develops earlier in the fall and disappears later in the spring than in a younger needle. This is related to differences in the depth of shoot dormancy.

6. In order to obtain reliable information on the character of seasonal changes, study of fixed material must be supplemented by in vivo observations, special care being taken to preserve the maximum number of cells in a living cell. For evaluation of these changes according to chloroplast condition, only living cells are suitable. Vaseline oil has proven an extremely satisfactory neutral medium for these observations,

7. The condition of agglutination observed by some workers in leaves and cortical tissues of various woody species was not detected in spruce needles.

8. Young developing plastids of new growth are considerably smaller than chloroplasts of old needles and are irregularly disposed in the cell, being found chiefly around the nucleus and to a very small extent in the cytoplasm between vacuoles. These plastids photosynthesize, as is evidenced by the presence of starch.

9. In a growing needle on new growth, the main type of nuclear division is amitosis.

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THE EFFECT OF LIGHT ON THE LIPOPROTEIN COMPLEX OF PLASTIDS

O. P. Osipova

K. A. Timiryazev Institute of Plant Physiology,
Academy of Sciences of the USSR, Moscow
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Utilization of the electron microscope in combination with refined techniques of perparating ultrathin sections has enabled investigators to determine minute changes in structure of the photosynthetic apparatus in relation to light conditions. It has been shown that the regular lamellar structure of green algae is peculiar to forms grown in the light. In the dark, however, there is a disruption of this lamellar condition which is concurrent with disappearance of chlorophyll [1,3]. It has been demonstrated for higher plants that leaf greening is associated with establishment of the layered structure and formation of grana [4-6].

This close connection between the presence of chlorophyll and a strict lamellar structure in algae on the one hand, and grana formation in higher plants on the other, is of great interest from the point of view of the function and condition of chlorophyll in the living cell.

Data showing that changes in structure of the photosynthetic apparatus entail definite quantitative changes in the lipoprotein complex of plastids are being accumulated in the literature.

De-Deken-Grenson [7] showed an increase in plastid protein during greening of etiolated chicory leaves; this was accompanied by an increase in plastid size and by appearance of a granular structure.

Of interest are the data of Lyttlton et al. [8, 9], showing that leaf tissues capable of photosynthesis contain a definite percentage of a water-soluble protein with a sedimentation constant, S_{20}, of 16-18. It was found that the amount of this protein fraction increases steadily as the etiolated leaves are illuminated, i. e., as the photosynthetic apparatus begins to function. The data of Lyttlton and T'so [10] showing that 70% of this fraction is localized in the chloroplasts attest to a close relation between the processes of photosynthesis and of protein formation. Because of its ready solubility in water, it can be assumed that the protein formed is not a component of the lamellar protein, this being, in all probability, a lipoprotein. Possibly it is that protein to which Nichiporovich [11,12] has attributed a special function peculiar to photosynthesizing tissues.

A study of Racusen and Aronoff [13] touching on qualitative changes in proteins of photosynthesizing tissues showed that there is an increase in rate of formation of aromatic and branched amino acids (phenylalanine, tyrosine, and leucines) in the light and that these are subsequently incorporated into leaf protein.

Amino acid synthesis in the light does not depend primarily on products of glycolysis but, according to the data of Andreev [14-16], probably involves intermediate products of photosynthesis, since the type of amino acid synthesized is reflected in protein of the green leaf, particularly of the chloroplasts.

Extensive data collected by Sisakyan [17, 18] testify to a qualitative reconstruction of chloroplast protein during photosynthesis which is instrumental in de novo formation of catalytic protein.

Increase in electrochemical mobility of plastid protein under the influence of light also attests to a qualitative change under these conditions [19].

Another component of chloroplasts which plays no small role in their structural organization, lipid, has received very little attention. Even in this case, however, there are some data on qualitative differences between lipids of achlorophyllous plastids, i.e., those which do not photosynthesize, and chloroplast lipids. Cromblie [20] has shown, for example, that the fatty acid fraction of white and green leaves of a number of plants, and also that of green and etiolated pea leaves, contains unsaturated acids; these latter comprised a higher proportion of the total fatty acid fraction in green tissues, with linoleic acid being especially prominent. Fats of non-green tissues and etiolated leaves were more saturated. Of interest in this connection is the study of Kobyakova [21], which showed an activating effect of light on lipoxidase, an enzyme involved in metabolism of unsaturated fatty acids.

It may be concluded therefore, although the data are still fragmentary, that protein and lipid of plastids are qualitatively altered in some manner under the influence of light. The presence of chlorophyll, which ensures absorption of light energy and its storage in prod-

TABLE 1. Content of Chlorophyll, Protein, Lipids, and Bromine (in mg per 100 mg dried plastids)

light regime employed	chlorophyll	protein	lipids	amt. of Br taken up
I. natural daylight	5.08	51.2	17.1	48.6
II. continuous light + 48 hr darkness	4.42	51.0	19.2	43.2
III. continuous darkness	0	54.5	18.1	30.7
IV. continuous darkness + 24 hr natural daylight	2.17	52.7	17.5	35.6
V. continuous darkness + 48 hr natural daylight	3.29	55.9	15.5	39.1

ucts of photosynthesis, is probably responsible for such alterations.

It is the purpose of this study to determine the qualitative characteristics of proteins and lipids of plastids formed in the dark, when there is no photosynthesis, and in the light.

Experimental material consisted of 10- to 12-day-old corn seedlings raised in a growing shed under the following light regimes: 1) natural daylight; 2) natural daylight + 48 hours of darkness; 3) continous darkness; 4 and 5) continuous darkness + 24 hours or 48 hours of natural day and night.

Seedlings were ground with cold 0.5M sucrose in a meat grinder, and the slurry was pressed through a double layer of linen. The solid residue was discarded and the suspension subjected to fractional centrifugation. First it was centrifuged 3 min at 1500 rpm, and the supernatant was then centrifuged 10 min at 3500 rpm. The precipitate was thoroughly washed once with cold sucrose and then examined microscopically. Examination revealed intact chloroplasts or leucoplasts, depending on whether the starting material was green or etiolated. Sucrose absorbed on the plastids was removed by a single washing with cold water.

Lyophilized plastids were assayed for chlorophyll; lipids, which were extracted together with chlorophyll by cold acetone; protein; and unsaturated compounds. The latter were determined by the micromethod of Wild [22].

The results of the analysis are presented in Table 1.

The data in Table 1 show that the amount of chlorophyll varies with light regime employed, while the content of other structural components bound with chlorophyll remains about the same.

The amount of Br absorbed by plastids, which is a measure of level of unsaturated compounds, was directly dependent on light regime. Chloroplasts isolated from leaves grown in natural daylight took up 48.6 mg, and those from leaves grown in continous light and then kept in the dark 48 hours took up 43.2 mg. Leucoplasts of etiolated leaves took up 30.7 mg; with a supplementary light period of 24 hours, they took up 35.6 mg, and with one of 48 hours, they took up 39.1 mg.

This direct relation between the amount of compounds with double bonds and the light regime suggests that photosynthesis plays a special role in their formation. In this case, light energy absorbed by chlorophyll is utilized in synthesis of compounds with double bonds (C=C), which requires, as is well known, a greater energy expenditure than does synthesis of single bonds (C−C). Because of their large potential chemical energy, unsaturated compounds are able to fulfill a definite function in chloroplasts. According to the hypothesis of Boichenko and Saenko [23], they can serve as transient acceptors of carbon dioxide, forming a loose union with it during photosynthesis.

It was shown experimentally by Holman and Widmer [24], using cardiac muscle mitochondria, that unsaturated compounds can participate in oxidation processes. A correlation of oxidase activity of the mitochondria with the presence of unsaturated compounds was found. It is assumed that such compounds may either be oxidase cofactors or may be an integral part of the enzyme.

In order to characterize the protein component of plastids formed under various light regimes, their amino acid content was determined chromatographically. The stroma was hydrolyzed with 6N HCl at 103-105° for 24 hours, having first been freed of pigments and lipids. HCl was removed by fivefold concentration on a water bath. A definite volume of hydrolyzate, containing 60-70 mg protein, was applied in a line to chromatograph paper and resolved in an n-butanol−acetic acid−water (4:1:5) mixture, with several passages of solvent through the paper. The procedure giving the best resolution was established in accordance with the type and quality of paper used. In addition, separate regimes were employed for three groups of amino acids differing in R_f values: I) lysine, histidine, arginine; II) asparagine, serine, glycine, threonine, III) alanine, tyrosine, valine + methionine, phenylalanine, leucine.

TABLE 2. Proteins of Plastids Isolated from Leaves Grown under Various Light Regimes (in % of protein component)

Amino acids	Natural daylight	Natural daylight + 48 hours darkness	Darkness	Darkness + 48 hours natural daylight
cystine	1.45	1.30	1.34	—
lysine	4.33	4.55	4.76	4.61
histidine	1.82	2.04	1.73	1.95
arginine	5.57	5.95	6.38	6.15
aspartic acid	6.73	6.79	6.50	6.83
serine	4.63	4.24	4.24	4.28
glycine	4.70	4.47	3.66	3.89
glutamic acid	7.67	7.61	7.78	7.83
threonine	5.41	4.91	4.85	5.08
alanine	5.49	5.23	5.17	5.26
tyrosine	4.30	4.17	3.53	3.99
valine + methionine	7.25	7.37	7.36	7.36
phenylalanine	5.49	5.65	3.97	5.04
leucines	11.67	10.20	9.41	10.09

Chromatograms were treated with a solution of ninhydrin in acetone in the presence of glacial acetic acid and cadmium acetate [25]. They were dried at room temperature for 30 min, and then placed in a chamber over H_2SO_4 and left overnight. Spots were outlined with a pencil, cut out and divided into small pieces, and eluted with methanol at room temperature for one hour. Optical density of the alcoholic solution was determined on an FKM colorimeter with a green filter against an eluate of a piece of paper cut from a blank part of the chromatogram. A mixture of amino acids of known concentrations in proportions approximately the same as those of the protein under study was chromatographed at the same time under the same conditions. Calculations were based on extinctions of the standard mixture and the hydrolyzate. Analyses were replicated five times. For some amino acids (lysine, histidine, arginine, glycine threonine, alanine, tyrosine, valine + methionine, phenylalanine, leucine), the error did not exceed 5%. For amino acids difficult to separate (asparagine, serine, glycine), the error could go as high as 7-10%.

Results are presented in Table 2.

They show that the component amino acids are the same for all proteins studied, but the relative proportions of individual acids are substantially altered with a change in light regime. Tyrosine, phenylalanine, leucines, glycine, arginine, and lysine showed especially marked variations in amount. Proteins of chloroplasts, in contrast to those of leucoplasts, contain relatively more tyrosine, phenylalanine, leucines, and glycine, the amounts of these compounds being 22, 38, 24, and 28% higher respectively. In leucoplast proteins, on the other hand, there is somewhat more arginine (14% more) and lysine (10% more). Similar differences were found in cases of short-term action of light or dark. For example, in proteins of plastids isolated from etiolated leaves after a short period of illumination, there was an increase in tyrosine, phenylalanine, and leucines, i.e., in those amino acids which are more rapidly synthesized in light.

In proteins of chloroplasts isolated from green leaves which had been in darkness 48 hours, there was a decrease in the amount of leucines. Differences in the content of basic amino acids (lysine and arginine) observed with the short-term action of light or dark were similar to those found in continuous light as against continuous darkness, i.e., a decrease in the light.

It should be noted that the effect of a short-term exposure to light is much more marked than that of short-term exposure to darkness. Obviously protein alteration in the light is more rapid than in the dark.

The fact that chloroplast proteins differ from leucoplast proteins in a higher content of aromatic and branched amino acids suggests that synthesis of these compounds is more dependent on photosynthesis than that of other amino acids. Evidently light energy absorbed by chlorophyll is utilized in synthesis of amino acids of complex structure, and this requires a greater energy expenditure than does synthesis of simple amino acids. The subsequent incorporation of amino acids into a protein molecule also depends on light regime. The process of photosynthesis is therefore a prerequisite condition for creation of the qualitatively distinct protein peculiar to photosynthesizing tissues.

Althought a protein's amino acid composition does not completely determine its physical and biological properties, it does affect them to a significant degree. For example, a higher content of amino acids with aromatic and branched structure evidently increases the hydrophobic properties of the protein, thereby rendering it more susceptible to combination with pigments and lipids. This property of a protein must be of critical importance in increasing the heterogeneity of the chloroplast as lamellae are being formed. The increased amounts of basic amino acids (lysine and arginine) in leucoplast protein enchance its hydrophilic properties.

SUMMARY

1. Changes in light regime lead not only to characteristic changes in plastid structure, but also to a qualitative alteration in lipoprotein complexes.

2. Amounts of substances of a predominantly lipid nature with double bonds in plastids increase in light.

3. Chloroplast proteins differ from leucoplast proteins in a higher content of tyrosine, phenylalanine, leucines, and glycine, and in a somewhat lower content of basic amino acids (lysine and arginine).

Other amino acids in the proteins studied show substantially no change with a change in light regime.

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ON THE EXTRACTABILITY OF CHLOROPHYLL FROM LEAVES BY A MIXTURE OF POLAR AND NONPOLAR SOLVENTS

D. I. Sapozhnikov and S. A. Chernomorskii

V. L. Komarov Botanical Institute, Academy of Sciences of the USSR, Leningrad
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It is well known that polar solvents (alcohol, acetone, etc.) easily and completely extract chlorophylls from green leaves. On the other hand, it is usually impossible to extract them with such nonpolar solvents as hexane or petroleum ether. In cases where small amounts of chlorophyll are extracted by these solvents, a surface-active agent of polar structure is present [1-3].

This study has been devoted to the subject of the extractability of chlorophyll by mixtures of polar and nonpolar solvents in various ratios. Information obtained in such a study might throw some light on the condition of chlorophyll in the plastid.

MATERIALS AND METHODS

The polar solvent employed was ethyl alcohol (96%), and the nonpolar solvent, petroleum ether (b. p. 45-70°). The following concentrations of alcohol in petroleum ether were studied: 0.1; 0.2; 0.3; 0.4; 0.5; 0.6; 0.7; 0.8; 0.9; 1.0; 1.1; 1.2; 1.3; 1.4%.

A 50-100 mg portion of leaves was ground with the solvent in a porcelain dish at room temperature, about 18°. The solvent was added in small aliquots, the tissue being ground 30-40 seconds after each addition. After each grinding, the solvent was poured off onto a glass filter before fresh solvent was added. Grinding was discontinued when fluorescence could no longer be detected. Ordinarily the grinding process required not more than 10-20 minutes. The final residue in the dish was extracted with a 3:1 mixture of acetone and alcohol.

Chlorophyll was quantitatively assayed on an FÉK-M colorimeter. The ratio of chlorophyll extracted by a given solvent mixture (C_e) to the total amount in the sample ($C_t = C_e + C_r$, where C_r is the amount in the solid residue) was taken as a measure of the effectiveness of the given solvent mixture in extraction.

Leaves of 40 plant species were investigated. A list of these species together with the appropriate figure numbers and curve designations is presented in the table.

Ordinarily, leaves analyzed were freshly collected from plants in the greenhouse of the Botanical Institute of the USSR Academy of Sciences. Leaves of some

List of Plants Investigated

Plant species	Figure No.	Curves	Plant species	Figure No.	Curves
Pelargonium zonale	1	—	Ligustrum vulgare	2C	III
Aucuba japonica	2A	I	Heritiera Fischeri	Ditto	Ditto
Thuja occidentalis	Ditto	Ditto	Coleus Blumei	»	»
Cupressus lusitanica	»	II	Taraxacum officinale	»	IV
Peperomia tithymaloides	»	»	Philodendron corsinianum	»	V
Clivia nobilis	»	III	Syringa vulgaris	2D	I
Philodendron oxicardium	»	Ditto	Aloe arborescens	Ditto	Ditto
Tradescantia virginiana	»	»	Aegopodium podagraria	»	II
Crinum asiaticum	»	»	Philadelphus coronarius	»	III
Acer laevigatum	2B	I	Alocasia odora	»	IV
Elaeis guineensis	Ditto	Ditto	Aralia spinosa	3	I
Aspidistra elatior	»	II	Vallisneria spiralis	Ditto	Ditto
Vitis voinieriana	»	Ditto	Fuchsia gracilis	»	»
Ruscus aculeatus	»	III	Daphne indica	»	»
Ficus elastica	»	Ditto	Antiaris toxicaria	»	»
Asparagus plumosus	»	IV	Salvinia natans	»	II
Dracaena Draco	»	Ditto	Dryopteris filixamas	»	Ditto
Dorstenia contrajerva	»	»	Echeveria glauca	»	»
Nymphaea stellata	2C	I	Cyclamen persicum	»	»
Phoenix dactylifera	Ditto	II	Chamaerops humilis	»	»

plants (gout weed, jasmine, lilac, and dandelion) were collected in the Botanical Institute gardens in the summer and analyzed in a dry condition. Succulent leaves were first pressed lightly between sheets of filter paper.

RESULTS

The dependence of amount of chlorophyll extracted from ground leaves on relative amount of alcohol in the solvent is graphically shown in Fig. 1 (plant, Pelargonium zonale).

As the figure shows, percent chlorophyll extracted increases with an increase in alcohol concentration. It is not hard to see that the curve is divided into two distinct parts, with a very marked shoulder at the point of inflection. One arm lies between 0.1 and 0.5% alcohol, and corresponds to 50% extraction. The other arm begins at 0.5% alcohol and represents further increase in extractability; complete extraction occurs at 1.0% alcohol.

Several " cardinal " points may therefore be distinguished: 1) the point corresponding to an alcohol concentration at which extraction begins; 2) the point at which inflection is beginning; 3) the point of inflection, at which the second arm begins; 4) the point at which complete extraction occurs.

In Fig. 2 are presented curves obtained with 29 plant species. They have been grouped according to the position of the third cardinal point, the point of inflection.

As Fig. 2 shows, these species can be divided into four groups on the basis of the alcohol concentration at which the second rise occurs. In 80% of the species represented by the curves of Figs. 1 and 2, the inflection point lies between 0.3 and 0.6% alcohol (Fig. 2, A, B, C), and in 20% of them it is at 0.8% (Fig. 2, D). The various curves in a single group differ in the position of the first and second cardinal points.

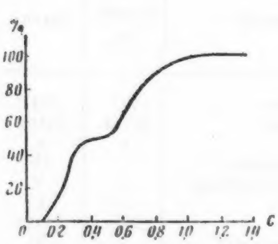

Fig. 1. Chlorophyll extraction curve for Pelargonium zonale leaves. Figures along the abscissa, concentration of alcohol in petroleum ether (in % by volume); figures along the ordinate, percent chlorophyll extracted.

In ten of the 40 species studied, the extractability curves differ markedly from those of Figs. 1 and 2 (Fig. 3), no inflection point being present. Curve 1 shows that in one group of plants extraction began at 0.1% alcohol, while curve 2 represents a group in which extraction began at 0.5%. For all species, complete extraction was effected by 1.0-1.4%.

An attempt was made to elucidate the phenomenon of the second rise (inflection point), which was observed in the majority of species, by extracting with a mixture of alcohol and petroleum ether leaves of gout weed, dandelion, and jasmine* before and after heating according to Sapozhnikov's method [4].

Results of these analyses are presented in Fig. 4, which shows 50% extraction with pure petroleum ether, and complete extraction at an alcohol concentration as low as 0.6-0.8%.

DISCUSSION

Analysis of the curves of Figs. 1 and 2, A-D, provides a basis for the assumption that in these plants binding of chlorophyll to protein is of two types. These data are in accord with data previously obtained in this laboratory [5] on the effect of heating leaves on extractability of chlorophyll by nonpolar solvents. It was shown that after short exposures to high temperature, some of the chlorophyll could be extracted with pure petroleum ether, while the remainder could only be extracted by polar solvents.

If it is assumed that we are dealing with chlorophyll in two forms, one absorbed to protein, and the other more closely combined to protein in the form of chromolipoprotein, then our findings may be interpreted as follows: Low concentrations (0.3–0.8%) of polar solvents induce desorption of chlorophyll and its passage into solution, but are unable to denature lipoprotein, to which the remainder of the cholrophyll is closely bound.

The fact that the alcohol concentration at which the inflection point occurs varies quite widely from species to species (0.3-0.8%) is in our opinion a consequence of differing amounts of surface-active materials present in the various species.

This explanation finds support in the character of the curves of Fig. 2. Those of Fig. 2, A and 2, B have an extremely steep slope to the inflection point, while those of 2, C and 2, D have gentler slopes. It is interesting to note that among the plants represented by curves in Fig. 2, A are Thuja and Cupressus, which contain considerable amounts of surface-active materials; in plants represented in Fig. 2, D, these materials are evidently small in amount.

The extraction curves obtained for ten species (Fig. 3, 1 and 2) can be rationalized by the assumption either that there is only one form of chlorophyll present, or that, as seems more probable, both forms are present, and one of them occurs in amounts too small to be de-

*See note on page 549.

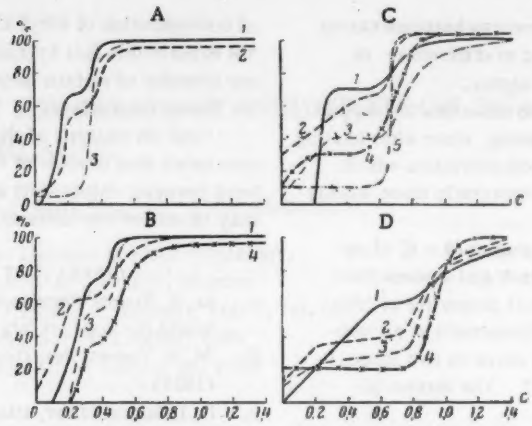

Fig. 2. Chlorophyll extraction curves for leaves of various plants. (For identity of plants, see table.) A) Plants with inflection point at 0.3% alcohol; B) plants with inflection point at 0.4% alcohol; C) plants with inflection point at 0.6% alcohol; D) plants with inflection point at 0.8% alcohol. 1, 2, 3, 4) Groups of plants made up according to differences in position of the first, second and fourth cardinal points.

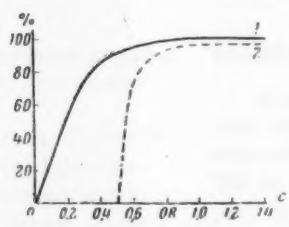

Fig. 3. Chlorophyll extraction curves with no inflection point, characteristic of certain plants. 1) Plants in which extraction begins at about 0.1%; 2) plants in which extraction begins at 0.5%.

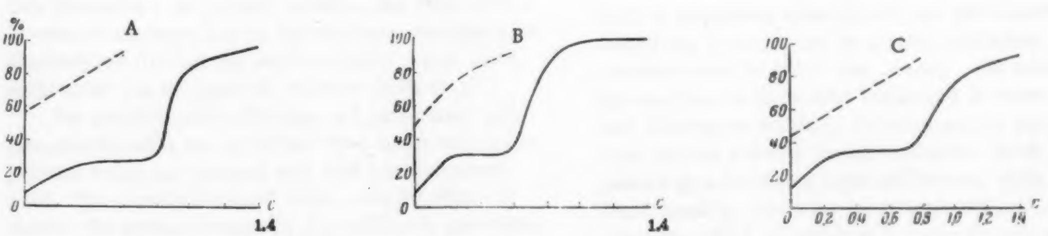

Fig. 4. Chlorophyll extraction curves for leaves of gout weed, jasmine, lilac, and dandelion,† before and after heating.

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†Individual graphs and curves unlabeled in Russian. Translation of plant names in text and figure caption follows Russian word order.

tected by our method. The differences between curves 1 and 2 are most probably related to differences in amounts of native surface-active agents.

Data on extraction before and after heating support our hypothesis of two types of binding, since after heating there is complete extraction at concentrations which release ordinarily only the first, apparently more weakly bound form.

On the basis of a number of studies [6 - 8] of optical properties of leaves (absorption and fluorescence spectra), and also of photochemical properties of chlorophyll bound in various forms, Krasnovskii et al. conclude that chlorophyll in plastids exists in two forms, "monomeric" and "aggregated." The former is fluorescent, and the latter is not.

It appears probable that the two forms observed in our experiment are to a certain extent analogous to those of Krasnovskii, though most likely they are not identical.

SUMMARY

The extractability of chlorophyll from green leaves by employing a mixture of nonpolar solvent and various concentrations of a polar solvent was investigated. It is demonstrated that in most of the investigated plants the extractability curves divide into two branches. In various plants the points of origin of the second branch (discontinuity point of the derivative) lie in a broad region of concentration of ethyl alcohol in petroleum ether. We explain this fact by assuming the existence of various amounts of surface-active substances belonging to the leaves themselves.

From an analysis of the curves obtained it can be concluded that in most of the investigated plants the bond between chlorophyll and proteins in the leaves may be one of two different types.

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THE EFFECT OF RESPIRATORY INHIBITORS ON HEAT RESISTANCE IN PLANTS

N. S. Petinov and Yu. G. Molotkovskii

K. A. Timiryazev Institute of Plant Physiology, Academy of Sciences of the USSR, Moscow
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Our previous studies [1,2] have established the dependence of plant heat resistance on respiratory processes. This study is an attempt to elucidate the mechanism of this dependence, there being a wide range of alternative means for coupling respiration to a given process. Such an attempt necessitates a search for the basic link in the chain of interrelated metabolic processes which, by virtue of its particular sensitivity to heat, determines the resistance of plants to high temperatures.

In this study respiratory inhibitors were used as tools to determine the relationship of heat resistance to various aspects of the respiratory process.

METHODS

The following plants were used: Mammoth tobacco, Gray Volzhskaya pumpkin, Sterling corn, Zelenka sunflower.

Plants were grown in transplanting flats at 25° and 60% relative humidity under continuous artificial illumination. For experiments leaves of the middle nodes of 3- to 5-week-old seedlings were selected. Excised leaves were heated in the absence of light in hermetically sealed chambers immersed in a Heppler ultrathermostat. Assessment of injury resulting from exposure to high temperature was made by the method of plasmolysis. Sections of epidermis were first stained with neutral red, then placed in a 1M sucrose solution, and then, after a certain time, depending on the material, examined for plasmolyzed (living) and unplasmolyzed (dead) cells. Respiration was measured in Warburg flasks at 30°.

For determination of amino and ammonium nitrogen, the material was extracted three times with water, proteins being precipitated with cold trichloroacetic acid. The extract obtained after centrifugation was assayed for amino nitrogen by Tsuverkalov's gasometric method. Ammonium nitrogen was determined by Conway's microdiffusion method with a subsequent nesslerization and colorimetric analysis. Inorganic phosphorus was determined according to the method of Fiske and Subbarow.

Amino acids were subjected to chromatographic analysis according to Boyarkin [3].

The following respiratory inhibitors were used: potassium cyanide, 1×10^{-3}M—inhibits all metal-containing oxidases; sodium arsenite, 1.6×10^{-3}M—inhibits oxidation of α-ketoglutaric acid in the Krebs cycle; monofluoroacetic acid, 0.02 - 0.04M—a structural analogue of acetic acid which condenses with oxalacetic acid to give monofluorocitric acid, this being incapable of further conversion by aconitase; 2,4-dinitrophenol (DNP), 4×10^{-4}M—uncouples oxidation from phosphorylation.

Inhibitor solutions were introduced into leaves by vacuum infiltration.

RESULTS *

The Effect of Respiratory Inhibitors on Heat Resistance

Respiration measurements were made immediately after infiltration of inhibitors. In every case there was a noticeable reduction in oxygen absorption (Table 1). The amount of this reduction was somewhat dependent on plant age, being progressively lower with time. Extreme values obtained for inhibition of respiration are presented in Table 1. It is characteristic that when enzyme systems are being inhibited (arsenite, cyanide) the degree of inhibition changes with age. Where there is interference with substrate supply (monofluoroacetate), degree of inhibition varies but little with age. Possibly there is a shift in respiratory systems with age and a decline in sensitivity of respiration to specific inhibitors. The proviso should be made that among the various plant species there is the widest variability in sensitivity to one inhibitor or another. For example, in pumpkin, cyanide induces a stable rise in respiration which is accompanied by accelerated sugar utilization, while in cucumber it inhibits respiration by at least 80%. Henceforth, when the effect of inhibitors is under discussion their suppressive action is meant.

* C—leaves infiltrated with water, unheated; C_h—leaves infiltrated with water, heated; E—leaves infiltrated with inhibitor, unheated; E_h—leaves infiltrated with inhibitor, heated.

TABLE 1. Effect of Respiratory Inhibitors on Rate of Oxygen Absorption in Various Plant Species (in ml O_2 per g tissue absorbed in 1 hour)

treatment group		percent
C	E	inhibition
sunflower; inhibitor, 1.6×10^{-3}M arsenite		
102.2	59.5	42
80.5	63.0	22
tobacco; inhibitor, 1×10^{-3}M cyanide		
142.3	57.6	60
327.0	217.0	34
pumpkin; inhibitor, 0.04M fluoroacetate		
575.0	433.0	25
371.7	296.0	22

Inhibition of respiration leads to a marked decline in heat resistance. Exposure to inhibitors rendered leaves susceptible to heat, and lethal injury occurred upon 2-3 hour heating at temperatures 5-9° lower than if there had been no exposure to inhibitors.

For example, irreversible damage to control pumpkin leaves (infiltrated with water) occurs with a 3-hour heating at 51-52°. Leaves infiltrated with inhibitors are killed at 45°. After a 3-hour heating, they are brown in color, their surface is covered with drops of cell sap, and they have a "cooked" appearance. Not a single plasmolyzed cell can be found after exposure of sections to sucrose solutions. In control samples, all cells are plasmolyzed, and neither immediately after the heating period nor after a 24-hour exposure to tap water at room temperature do the leaves exhibit signs of injury. The results of one such experiment are illustrated in Fig. 1. Similar results were obtained with the other inhibitors, including 2,4-dinitrophenol, in all the plants treated.

This effect of inhibitors on resistance cannot be attributed to an acceleration of their action by higher temperature. Cyanide inhibits tobacco leaf respiration by 40-60%, but the injured cells nonetheless remain alive for 48 or more hours. At the same time, cyanide-injured cells exposed to high temperature die within 1.5-2 hours. Evidently there is a marked rise in substrate and energy requirements with increased temperature.

Respiration and Amino Acid Synthesis at High Temperature

Upon heating, the amino acid content of plant tissues increases markedly. This was observed long ago and regarded as evidence that at high temperature the degradation of protein to amino acid is accelerated. The excessive rise of amino acid content in heat resistant plants remained, nevertheless, a puzzling fact. The paradox was resolved by the assumption that resistant plants respond to heat by a unique protective reaction—acceleration of proteolysis [4].

We have also found a perceptible rise in amino nitrogen with heating, but it does not exceed 60%, probably because of the small exposures to heat (Table 2). This accumulation of amino acids with heating is retarded by respiratory inhibitors.

The inhibitors most effective in this respect are arsenite and fluoroacetate, which interfere with the Krebs cycle. At the present time, it is believed that the reduction in amino acid content which accompanies inhibition of respiration by fluoroacetate [5] and arsenite [6] is associated with a retardation in amino acid synthesis. The keto acid deficiency which is consequent on inhibition of respiration leads to a decline in amino acid levels, since keto acids are substrates for amination reactions.

The retardation of amino acid synthesis by respiratory inhibitors is also manifested at normal temperatures, this being particularly true of DNP. Inasmuch as the ATP content of tissues is limited, the effect of DNP on synthetic processes can be easily demonstrated with relatively short exposure periods. The following experiment was set up to show the effect of arsenite and DNP on amino acid synthesis.

Bean leaves were infiltrated with 0.05M NH_4NO_3+ 0.1M glucose (control) and the same mixture plus

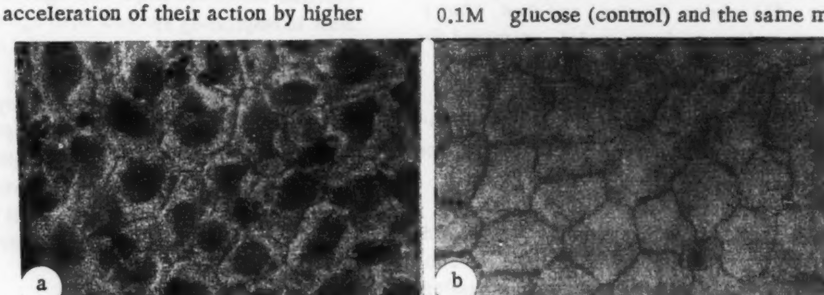

Fig. 1. Photomicrograph of pumpkin leaf epidermis after a 3-hour heating at 45°. Stained with neutral red, 10 min in 1M sucrose; a) leaves infiltrated with water prior to heating, all cells alive (plasmolyzed); b) leaves infiltrated with 0.04M fluoroacetate, all cells dead (no plasmolysis).

TABLE 2. The Effect of Respiratory Inhibitors on Amino Acid Synthesis at High Heat (in mg NH_2-N per 100 g tissue)

Plant	Inhibitor	Exposure		Treatment group			Δ NH_2–N
		hours	t. °C	C	C_h	E_h	
sunflower	arsenite	3	44	23.67	32.96	27.04	−5.92
corn	the same	4	45	—	11.72*	10.59	−1.13
barley	»	4	42	35.56	46.32	38.97	−7.35
pumpkin	»	3	45	—	32.38	24.56	−7.82
sunflower	fluoroacetate	3	44	20.61	30.5	24.67	−5.83
pumpkin	the same	3	48	—	47.57	38.14	−9.33
tobacco	DNP	2	42	—	20.0	17.14	−2.86
the same	the same	2	43	—	30.0	27.8	−2.2
»	cyanide	2	43	—	—	27.2	−2.8
»	DNP	2	43	15.65	16.21	15.09	−1.12
»	cyanide	2	43	—	—	15.09	−1.12

* Contains many amides.

10^{-3}M arsenite (experimental). Exposure period was 20 hours, temperature 20°. Tobacco leaves were infiltrated with water, 0.02M $(NH_4)_2CO_3$ (control), and 0.02M $(NH_4)_2CO_3 + 4 \times 10^{-4}$M DNP (experimental). Exposure period was 4 hours, temperature 20°.

The following results were obtained (in mg NH_2-N per 100 g tissue):

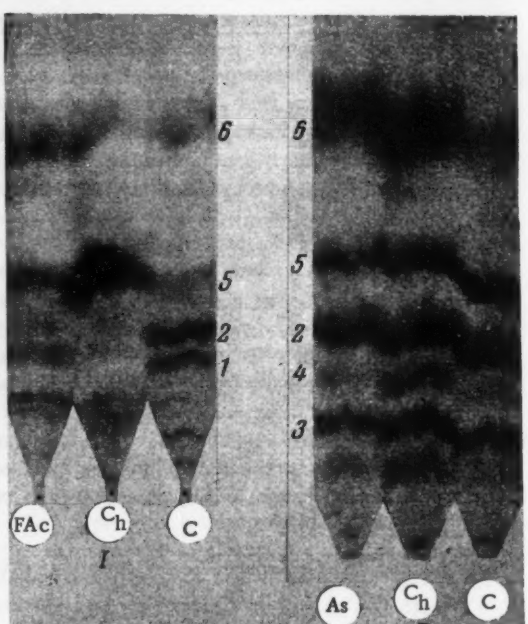

Fig. 2. Chromatogram of free amino acids. I) Pumpkin leaves: unheated (C), heated 3 hr at 45° (C_h), heated with a preliminary infiltration of 0.04M fluoroacetate (FAc); II) sunflower leaves: control (C), heated 3 hr at 44° (C_h), heated with infiltration of 10^{-3} M arsenite (As). 1) Aspartic acid; 2) glutamic acid; 3) asparagine; 4) glutamine; 5) alanine; 6) γ-aminobutyric acid.

Treatment group	H_2O	Control	Experimental
Bean	–	53.6	34.5
Tobacco	16.8	28.3	21.3

It is striking that with optimal temperature conditions, the decline in amino acid synthesis under the influence of inhibitors is greater than with high temperatures. Apparently the increase in amino nitrogen with strong heating can be attributed only in part to synthesis. A definite part of the amino acid complement represents proteolysis products which have not undergone oxidative deamination. Thus, during strong heating two coexisting pathways for conversion of nitrogenous substances are brought into play a regressive and a regenerative or, according to Aleksandrov's terminology [7], a reparative pathway.

It is difficult to determine the relative importance of the reparative pathway, since it is impossible to completely block it with the inhibitors under study. All known inhibitors can only slow down a given reaction, not stop it completely.

Additional proof of the existence of two pathways of amino acid conversion is supplied by data on changes in ammonia content of tobacco leaf tissues induced by inhibitors and high temperature (Table 3).

The amount of ammonia accumulated during strong heating is a resultant of two processes occurring at different rates —oxidative deamination and synthetic

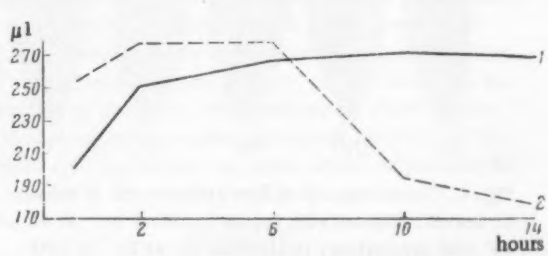

Fig. 3. Time course of tobacco leaf respiration under the influence of DNP: 1) infiltration with water; 2) DNP.

TABLE 3. Effect of Respiratory Inhibitors on Ammonia Formation in Tobacco Leaves during Strong Heating (in mg NH_3 - N per 100 g tissue)

Inhibitor	Exposure		Treatment group			ΔNH_3–N
	hours	t, °C	C	C_h	E_h	
DNP	2	42	—	400	1440	1040
the same	2	42	—	1424	1803	379
cyanide	2	42			2746	1322
DNP	2	43	—	2780	3640	860
cyanide	2	43			3670	890
DNP	2	49	2372	3164	3278	114

binding of ammonia in the form of amino acids and amides. Retardation of synthetic processes by inhibitors shifts the over-all balance in favor of the regressive pathway, which ultimately leads to an increase in ammonia. It is noteworthy that at high lethal temperatures (49°) inhibitors no longer have an additive effect in relation to ammonia formation.

Chromatographic analysis of the amino acid complement reveals that when leaves are strongly heated it is primarily glutamic acid, aspartic acid, their amides, alanine, and γ-aminobutyric acid, i.e., the chief amino acids involved in transamination reactions, which increase in amount (Fig. 2).

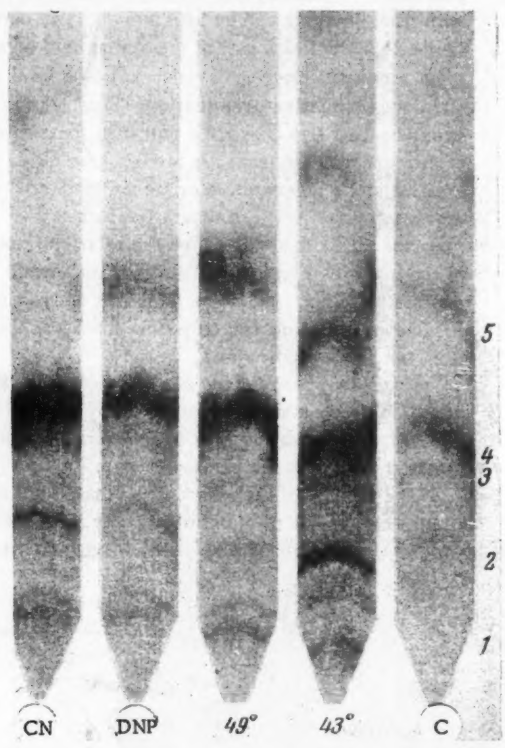

Fig. 4. Chromatogram of free amino acids of tobacco leaves. Control (C), leaves heated 2 hr at 43 and 49° with preliminary infiltration of 4×10^{-4} M DNP and 10^{-3}M cyanide (CN). 1) arginine; 2) glutamic acid; 3) unidentified amino acid; 4) alanine; 5) γ-aminobutyric acid.

With a preliminary exposure to inhibitors, the amount of these amino acids, with the exception of γ-aminobutyric acid, falls during heating. As a rule this decrease is especially marked in the case of glutamic acid and glutamine.

γ-Aminobutyric acid exhibits somewhat anomalous behavior, inasmuch as it steadily increases with heating, and when leaves are first infiltrated with inhibitors it increases still more. This compound is regarded as a derivative of glutamic acid [8], and also as a donor of amino groups and a carbon skeleton for protein synthesis [9]. Evidently the increase observed is related to atypical changes in amino acid metabolism.

Relationship of Heat Resistance to Oxidative Phosphorylation

In the course of inhibiting the over-all process of respiration, respiratory poisons ultimately bring about a nonspecific inhibition of oxidative phosphorylation [10]. This is particularly true of cyanide, which impedes electron transfer through the cytochrome system and thereby affects about 90% of ATP synthesis.

The reduction of heat resistance induced by respiratory inhibitors may therefore be related both to a deficiency in Krebs cycle substrates and to a decline in energy supply as a result of a sharp drop in ATP synthesis. Experiments with DNP have made it possible to elucidate the role of oxidative phosphorylation in the phenomenon of heat resistance.

The concentration employed, 4×10^{-4}M, stimulates respiration slightly and therefore is in the range of concentrations which affect oxidative phosphorylation most strongly.

The Effect of DNP on Tobacco Leaf Respiration ($\mu l\ O_2$):

t, °C, exposure 2 hr	Control	DNP
20	292.8	347.1
20	141.3	160.9
42	85.3	77.4
43	211.6	113.2
20	152.6	161.3
42	215.0	79.3

The effect of DNP on respiration develops with time. The original stimulation is considered to be a result of removal of the limiting effect of oxidative phosphory-

TABLE 4. Formation of Inorganic Phosphorus in Tobacco Leaves under the Influence of High Temperature and Inhibitors (in mg $P_{inorg.}$ per 100 g tissue)

Exposure No.	Exposure: hr	Exposure: t, °C	Treatment: control	Treatment: DNP	Treatment: KCN
1	3	20	19.9	12.4	10.3
	3	43	18.8	27.5	29.5
2	2	20	9.2	10.4	9.8
	2	42	9.2	18.4	18.0
3	2	20	14.2	—	—
	2	43	17.9	21.1	19.5
	2	49	22.2	—	—
4	2	20	14.1	—	—
	2	49	21.8	28.4	—

lation [11]. Inasmuch as the amount of phosphorus acceptors (ADP) is limited, oxidative processes, which are coupled to phosphorylative processes, cannot go at a rate exceeding that at which the acceptors are formed. With uncoupling, the controlling influence of a limiting amount of ADP is removed, and oxidation can proceed more rapidly without the synthesis of high-energy bonds of ATP. As ATP disappears, phosphorylation of respiratory substrate is gradually slowed down and then stopped, and the rate of respiration falls. This is illustrated by the time course curve of respiration under the influence of DNP (Fig. 3).

The respiration of DNP-treated leaves is markedly reduced with a two-hour exposure to high temperature, as shown in the graph. Evidently there is a significant increase in ATP destruction at high temperature, which ultimately leads to a fall in respiration.

The interrelationship between respiration and metabolism of organic phosphorus compounds may be judged with some accuracy according to changes in content of inorganic phosphorus ($P_{inorg.}$). With relatively short exposure periods, the changes observed may be considered as related to synthesis or destruction of labile organic phosphorus compounds.

As temperature is increased, the quantity of inorganic phosphorus released rises and reaches impressive levels at lethal temperatures (Table 4). This is direct evidence of rapid destruction of organic phosphorus compounds under these conditions. It is interesting to note that rate of $P_{inorg.}$ release does not increase with temperature in a linear fashion. In the range up to 42° there is even sometimes a decrease, but from 43° release is increasingly rapid.

Destruction of organic phosphorus compounds in DNP-infiltrated tissues is markedly enhanced even at comparatively low temperatures, around 40-43°, and the extent of this destruction compares with that in controls at 48-49°.

Identification of the free amino acids formed at high temperature and under the influence of various inhibitors showed that DNP and high lethal temperatures produce similar effects. Heating of DNP-treated leaves at 43° has exactly the same effect on amino acid composition as does heating of control leaves at 49° (Fig. 4). On the other hand, cyanide-treated leaves have an amino acid complement after heating which differs from that of control leaves. The most characteristic feature is the unvarying presence of γ-aminobutyric acid after exposure to high temperature or to DNP.

DISCUSSION

Inhibition of respiration by various inhibitors leads to a sharp decline in heat resistance, and this decline evidently depends on the depression of a number of synthetic processes. At present, the view that organisms actively resist unfavorable environmental conditions, including temperature [12,13], is being more and more widely accepted.

Plant heat resistance, even in its extreme manifestations (thermophiles of hot springs at 80°), is undoubtedly a property acquired in the course of evolution [14] which is associated with profound changes in metabolism. Even the relatively limited artificial adaptation of microorganisms to high temperature entails a reconstruction of the entire metabolic system, including a reconstruction of the enzymatic apparatus of respiration [15].

The unique role of respiration in resistance is determined by the necessity of maintaining vigorous counter measures to minimize heat injury. This involves primarily protein synthesis and synthesis of the amino acid precursors. Even a slight inhibition of respiration at high temperatures leads to a marked retardation of amino acid synthesis from products of regressive protein metabolism. The requirement for a flow of substrates for synthetic reactions and for energy to maintain these reactions, these being provided by respiratory activity, increases with increased temperatures. Thus, leaves of the most varied plants can remain alive with impaired respiration for a few days, but exposure to high temperature results in almost immediate death.

Experiments with DNP furnish a basis for the assumption that phosphorylative oxidation is the component of respiration most severely damaged by high temperature, and that it plays a dominant role in heat resistance. This is corroborated by the relative similarity in effect of high temperature and of DNP. In both cases, the destruction of phosphate esters induces an initial stimulation of respiration with a subsequent decline, and the amino acid spectra are the same, which emphasizes the similarity in destruction processes.

SUMMARY

The effect of a number of respiratory inhibitors (KCN, monofluoroacetate, arsenite, 2,4-dinitrophenol) on heat resistance of leaf tissues of various plants has

been investigated. It has been established that the inhibition induced involves a pronounced decline in resistance of tissues to overheating. Proteolysis of proteins is intensified, and ammonia accumulates in the tissues. Furthermore, amino acid synthesis (especially of alanine, glutamic and aspartic acids and their amides) ceases, and abnormal changes in amino acid metabolism take place (accumulation of large amounts of γ-aminobutyric acid).

It is concluded that protective reactions against heat exist in plants. Their essence is resynthesis of proteins destroyed by high temperature. The source of active metabolites and energy for the resynthesis is respiration.

Heat and 2,4-dinitrophenol are found to have similar effects on some aspects of plant metabolism (accumulation of inorganic phosphorus, respiration, formation of amino acids). On this basis it is concluded that oxidative phosphorylation may be important for heat resistance in plants.

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EFFECT AND AFTER-EFFECT OF ANAEROBIC CONDITIONS ON WATER REGIME AND RESPIRATION OF PLANTS

G. M. Grineva

K. A. Timiryazev Institute of Plant Physiology, Academy of Sciences of the USSR, Moscow

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The problem of physiological activity of plants, and particularly of water regime during insufficient aeration, is important not only for the study of anaerobic metabolism, but also has a practical significance, such as growing of plants on marshy or over-inundated soils.

It has been observed that retardation or cessation of ion absorption by roots occurs when oxygen is absent, and occasionally retardation or cessation of ion discharge into the external medium [1].

From data in the literature it is known that the plant tissues or the entire plants decrease in their capacity to absorb water during oxygen deficiency or in an anaerobic condition [2-5]. Semikhatova [6] had observed retardation of water-withholding capacity in oat coleoptiles when oxygen respiration was stopped. Oota [7], studying Vigna embryos, has concluded that absorption of water is a process closely connected with aerobic metabolism. The accumulation of labeled calcium was observed in aquatic plants during anaerobiosis, the calcium being rapidly used up by the plant when transferred into a normal medium [8]. The author concludes that oxygen evidently is necessary for transport of calcium from the tonoplast into the other parts of the cell. According to the observations of Khotyanovich [9], insufficient aeration of the soil, caused by flooding, had a negative effect on the entrance of water, nitrogen, phosphorus, and potassium into the roots of pine. The harmful effect was noted in plants during flooding and also after its termination. The time of flooding is of great significance. Plants flooded for one day usually resumed their normal living activity; two or three days of flooding caused notable permanent injury; five to six days of flooding was lethal for the plants [3]. The cessation of normal function of the roots in corn and wheat after prolonged anaerobiosis has also been noted by Bazyrina [10].

Therefore, the lack of oxygen is reflected in the functional activity of plants, decreasing the absorption of water and nutrient substances.

The present work is connected with the study of the direct effect of anaerobic conditions and their after-effect on the absorption of water, intensity of transpiration and respiration in young corn and sunflower plants.

OBJECTS AND METHODS OF INVESTIGATION

The experimental objects were young plants, with 2 or 3 leaves, of early-maturing corn, varieties IGAN-3 and Spasovskaya, and sunflower, varieties Saratovskii 169 and Zelenka 368. The plants were grown in the greenhouse in crocks filled with gravel and fed by Knop's nutrient mixture.

For studying the after-effect of anaerobic conditions we used plants of corn, variety IGAN-3, and sunflower, variety Zelenka 368. The plants, with thoroughly washed root systems, were placed into moist glass chambers with gaseous nitrogen, containing 0.6-0.8% oxygen.

Control plants were placed into the chamber with air. The exposure was varied in the experiment: 6, 12, 18, 24, and 42 hours in the darkness. In some cases the entire plants were placed into the chambers, and in others only the root system was subjected to the treatment. The whole plants were also treated by a gas mixture: 95% nitrogen and 5% oxygen.

After the exposure, the plants were taken out of the chambers and carefully dried with filter paper, and roots of 4 to 5 samples were placed into the volume-measuring devices of Sabinin-Kolosov for determination of rate of water absorption [11] taking place in the light, in the air, for 4 hours. Distilled water (22-24°) in the volume-measurer was previously aerated by blowing air. The experiments were done in four replicates. The results were calculated in milliliters of water per hour per cm^3 of roots. The rate of transpiration was determined by weighing flasks with the plants before and after the experiment, and was calculated per gram of dry weight of leaves per hour. Respiration of leaves and roots was determined in Warburg manometers after treatment, in $\mu l\ O_2$ per gram wet weight per hour. The control with air was carried out in every experiment.

The experiments with absorption of water and transpiration directly in the atmosphere without oxygen

TABLE 1. Respiration Rate in Plants after Treatment with Nitrogen (In μl O_2/hr/g wet weight)

plant	treatment	immed. after expt.		1 hr after expt.	
		absolute	in % of control	absolute	in % of control
sunflower, roots	after 6 hr in nitrogen	195.1	172.7	134.0	60.2
	in the air	113.0	100	223.4	100
sunflower, leaves	after 6 hr in nitrogen	416.7	227.2	352.7	77.7
	in the air	223.4	100	453.4	100
corn, roots	after 6 hr in nitrogen	154.1	103.4	259.1	103.7
	in the air	149.0	100	249.8	100
corn, leaves	after 6 hr in nitrogen	177.8	201.2	386.8	113.5
	in the air	78.4	100	340.5	100

were carried out with plants of corn, variety Spasovskaya, and sunflower, variety Saratovskii 169, in hermetically sealed plastic chambers into which the volume-measurers and flasks with plants were placed. Gaseous nitrogen, purified from oxygen, was blown through for 6 hours through the experimental chamber. Air was blown through the controls. Water purified from oxygen was used in the experimental chamber, while in the control the water was aerated. Relative humidity of the air in the chambers was 80-90%. The amount of oxygen in the nitrogen chamber did not exceed 0.4-0.6% in the course of the experiment.

EXPERIMENTAL

The experiments carried out directly in the atmosphere, without oxygen, have shown a considerable decrease in water-absorption capacity and transpiration rate both in corn and in sunflower. This is particularly clearly expressed in the latter. The absorption of water in the sunflower was 26.1%, while in corn it was 41.5% as compared to the control. An exposure of 18 hours generally showed the same results as one of 6 hours; because of this, we have concentrated on the latter.

After the experiment, the plants were slightly wilted in spite of a high air humidity in the chambers. Consequently, the possibility of decrease of water exchange as a result of tissue saturation with water is excluded. The absorption of oxygen in plants is considerably increased after anaerobic conditions (Table 1).

From the data of Table 1 it can be seen that the sunflower is more sensitive to the lack of oxygen than is corn, which to some degree resumes normal respiration after anaerobiosis. The greater resistance of corn to anaerobic conditions is thus connected with the respiratory process, which in turn determines the absorption of water. Evidently, this burst of respiration is not connected with a toxic action of nitrogen, since the substitution of nitrogen by the air after one hour sharply decreased the respiration rate.

The respiration rate of control plants in turn is considerably increased in the light. Experimental plants did not resume respiration to the normal degree, except for the corn roots.

This original burst and gradual decrease of respiration when air is replaced by nitrogen is observed also in the experiments with potato tubers [12,13,14]. The rate of CO_2 evolution in the air in apple after anaerobiosis was also noted by Blackman [15]. Similar changes in the respiration rate after treatment with nitrogen were also observed by other authors [16, 17]. The after-effect of anaerobiosis has caused, simultaneous with a fall in respiration, a decrease in sugar content and increase in lactic acid evolution [12, 13, 14].

Evidently the burst of respiration is connected with the increase in carbohydrate substrate and with utilization of accumulated lactate, which, being oxidized to pyruvate, may be used in respiration via the Kreb's cycle.

Anaerobic conditions, which interfere with metabolism, also lower the water-absorbing capacity of plants. In our experiments, water absorption was studied in relationship to the after-effect of anaerobic conditions.

In the experiments with whole plants of corn, which were placed into a nitrogen atmosphere during various periods of time, a strong increase in water absorption and transpiration was observed as a rule when the plants were transferred to air. After 6-hour anaerobiosis, the water absorption was increased twofold as compared to the control. Only after a day's exposure did the water absorption sharply decrease and fall below the control (Fig. 1, A I).

Increase of exposure to 42 hours has emphasized this relationship, evidently causing irreversible injury to functional activity. Respiration of leaves and roots, decreased as compared to the control, gave a burst only after a day's anaerobiosis. The sharp decrease in water absorption, along with the increase in respiration, evidently is connected with the breakdown of the exchange reactions of respiration. After a day's anaerobiosis the living activity of cell protoplasm is completely retained, which can be seen by the cell's capacity to plasmolyze (Fig. 2). Plants placed into water after a

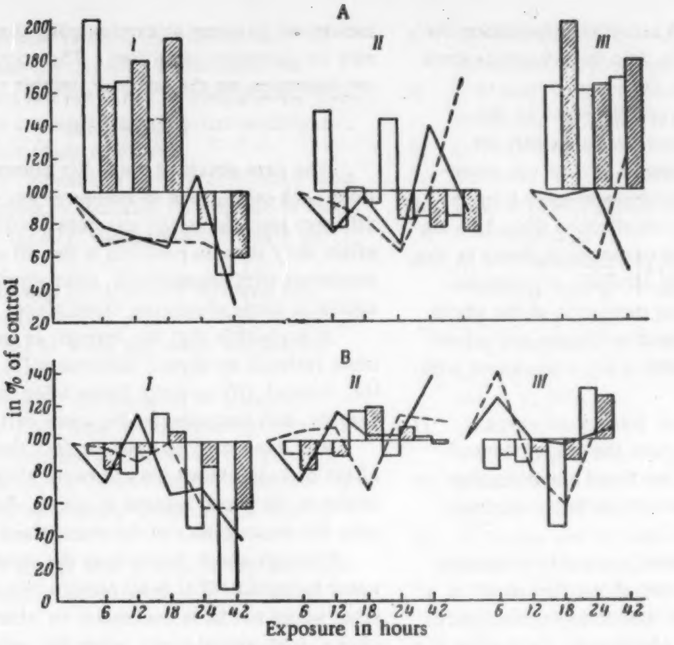

Fig. 1. Effect of anaerobic conditions on water absorption, transpiration and respiration rate in plants of corn (A) and sunflower (B).

I) Nitrogen treatment of the whole plant; II) Nitrogen treatment of root systems; III) Treatment of the whole plant by 95% nitrogen plus 5% oxygen. Nonshaded columns—absorption of water; shaded columns—transpiration rate; black curves—leaf respiratic dotted curves—root respiration.

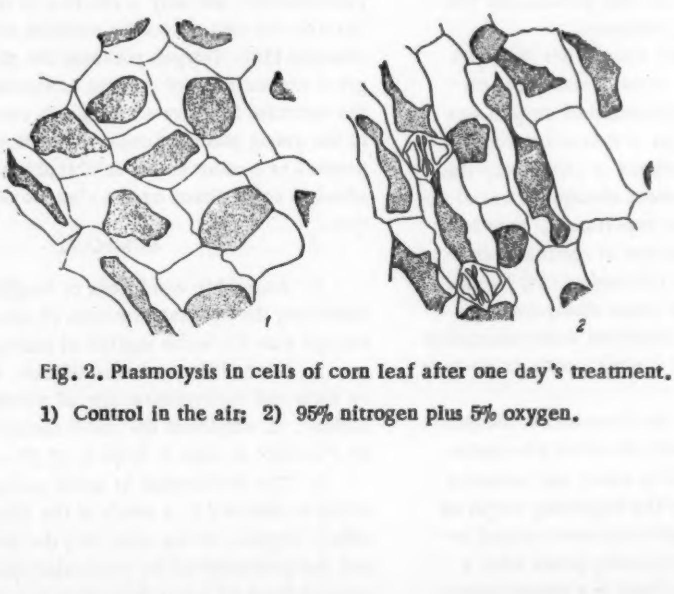

Fig. 2. Plasmolysis in cells of corn leaf after one day's treatment.

1) Control in the air; 2) 95% nitrogen plus 5% oxygen.

24-hour experiment have kept a normal appearance for several days, without our being able to distinguish them from control plants.

When only root systems were subjected to the treatment, we also found an increased capacity for water absorption. Water absorption in corn was represented by two-peaked curves with peaks after 6 hours and after 18 hours treatment with nitrogen (Fig. 1, A II); the broken rhythm of the water exchange is shown in the decrease in transpiration during all these experiments. Here, in the same way as in the treatment of the whole plant, the retardation of the water exchange and a burst of respiration were observed after a day's treatment with nitrogen.

With increase in the partial pressure of oxygen, i. e., placing the whole plants into the gas mixture of 95% nitrogen and 5% oxygen, we found a relationship similar to that which was shown in the first experiment (Fig. 1, A I).

Therefore, the after-effect of anaerobic conditions or a deficiency of aeration of corn shows first of all in the increase in water absorption and transpiration, and in decrease in the rate of oxygen absorption. Only after a day's anaerobiosis is there a breaking point in plants, when water absorption is sharply retarded and oxygen absorption is increased.

The second plant studied – sunflower – has shown slightly different relationships as compared with corn.

Anaerobic conditions for 6 and 12 hours caused a decrease in water-absorbing capacity and transpiration (Fig. 1, B I). It is interesting that 18-hour anaerobiosis increased water absorption, which fell again sharply after 24-hour exposure. The root respiration was correspondingly decreased during first exposures, reaching the control level toward an 18-hour period, and was sharply decreased by 24-hour exposure.

In the leaf, the respiratory process has the same direction, but the respiration curve is somewhat displaced in time. Maximum absorption of oxygen was observed after a 12-hour period of treatment (Fig. 1, B I).

In the experimental treatment in which only the root system was treated, the water absorption was of the same nature as of the previous experiment. Root respiration was retarded in the period of optimal water absorption (Fig. 1, B II). The mixture of 95% N_2 plus 5% O_2 favored the increase in water absorption only after a day's treatment. The minimal water absorption (to almost 40% of the control) occurs with an 18-hour treatment (Fig. 1, B III).

The transpiration rate in the experiment changed, in general, correspondingly with the water absorption.

Stimulation of respiration in aerial and subaerial organs was observed only after the beginning stages of treatment. The more prolonged treatment caused retardation of respiration, again forming peaks after a day's treatment. In sunflower there is a characteristic maximum in water absorption only after an 18-hour stay in anaerobic conditions. The comparatively shorter exposures, on the contrary, inhibit water exchange.

DISCUSSION

The data obtained show that plants greatly differing from each other, such as sunflower and corn, have a different response to the anaerobic conditions. One effect they show in common is the effect of one-day treatment with anaerobiosis, after which a sharp decrease in water absorption takes place.

It is possible that the cereals, as well as plants more resistant to oxygen deficiency [6], may reestablish normal life activity faster when insufficiently aerated, and compensate the water deficit.

It is interesting to note the fact that the after-effect of anaerobiosis may have an altogether opposite effect on the water regime of plant, when compared with the direct effect of the atmosphere lacking oxygen.

Through which forces does the plant regulate its water balance? If it is by osmotic forces, then the plant would not have decreased its absorption of water after a day's anaerobiosis, when the cells were viable, but the plant would have wilted. Also, the sharp inhibition of water absorption capacity, with the absence of oxygen respiration, indicates that osmotic forces are not the basic factor. It is true that some part of absorption is evidently connected either with osmosis, or with the energy of glycolysis, since the absorption does not altogether stop with the anaerobiosis. Evidently, the decrease in water absorption is connected with the breakdown of the general metabolism of plants, which also changes the respiratory system.

It is known that with the change of the oxygen participation, not only is the rate of respiration changed, but also the role played by oxidases in this process changed [18]. Oxygen prevents the plant from too great expenditures of organic substances. Evidently, the decrease in water exchange in anaerobic conditions in the young plants of corn and sunflower takes place in general as a result of the inhibited plant metabolism, which is conditioned by the absence of oxygen respiration.

SUMMARY

1. Anaerobic conditions or insufficient aeration, inhibiting the general direction of metabolic process, change also the water regime of plants.

2. In conditions of anaerobiosis, water absorption by roots and transpiration rate of plants are sharply inhibited. In sunflower the water absorption drops to 26.1% while in corn it drops to 41.5% of control value.

3. The stimulation of water exchange in plants, which is observed as a result of the anaerobiosis after-effect, depends on the time and the method of treatment and the properties of the particular species. The increased level of water absorption is connected usually

with 6- to 18-hour exposure in anaerobic conditions. After a 24-hour exposure the capacity to establish the normal water balance rapidly is lost in both plants. The longer exposures—for example, 42 hours—irreversibly inhibit the normal functioning of plants.

4. Corn is more resistant to oxygen deficiency than sunflower and is capable of restoring its respiration and water balance more rapidly when transferred into normal conditions.

5. Treatment of root systems with nitrogen has shown, in general, results similar to those obtained with the whole plant insofar as water exchange and respiration is concerned.

6. In all probability, the decrease in water exchange in anaerobic conditions or during oxygen deficiency is connected with the inhibition of the general metabolism, the energy of which cannot be utilized by the plant sufficiently effectively when oxygen respiration is absent.

In conclusion, I should like to express my gratitude to Professor N. S. Petinov for valuable advice during this work.

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PHYSIOLOGICAL CHARACTERISTICS OF GERMINATION OF RICE SEEDS

E. P. Aleshin

Kubanskaya Rice Experimental Station, Krasnodar
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With respect to methods of cultivation, rice occupies a very special position among other grain crops: Its germination takes place when the soil is flooded with water. In spite of this, there are no clear conceptions about the interrelationships between germinating grains of rice and the layer of water.

It is known that rice seeds germinate as well on moistened as on flooded substrate [1], since they require for germination only 1/500 as much oxygen as is needed by cotton [2]. It has been established that in water the rice epicotyl grows rather than the root. Because of these investigations, rice was contrasted with other cereals, while Kleb's hypothesis concerning the adaptation of rice seeds to the germination in water has been established and was confirmed in the work of a large number of authors; with this, it has been found that the respiratory coefficient in seeds germinating under water considerably exceeds unity [3]. With this in mind, it has been proposed that the exceptional capacity of rice for germination under anaerobic conditions is closely connected with unusually energetic fermentation [3,4]. Confirmation of this was found, as a number of authors have found a considerable amount of ethyl alcohol in the sprouts, as well as in the distilled water which is most frequently used for the establishment of anaerobiosis around the sprouts [6,7]. It is most noteworthy that these facts do not hold when seeds are germinated under conditions of sufficient aeration [3].

In connection with this proposal, one may consider that atmospheric oxygen is the regulator of the germination of rice seed. However, even the existence of such a conclusion does not allow us to judge about those physiological processes which take place in the germinating grain. Because of this, it seems to be very interesting to study the mechanism of germination of rice seeds, to understand the reasons of the high resistance of the young sprouts against flooding.

To clarify the internal conditions of germination, experiments were set up in which rice seeds, variety VROS 3716, were germinated on moistened filter paper and also flooded with 15 cm layer of freshly boiled distilled water. During germination of seeds, measurements were made of the rate of respiration, activity of oxidases and dehydrogenases, changes of Eh and pH of sprouts, as well as growth of embryo, content of vitamins B_1 and C, and heteroaxin. Respiration and activity of oxidases were measured gasometrically in a Warburg apparatus [8]. As inhibitors of metal-containing oxidases, we used sodium azide in a concentration of 10^{-3} M and sodium diethyldithiocarbamate in concentration of 2×10^{-3} M. The total dehydrogenase activity was determined after Thunberg [8], pH and Eh were determined by a potentiometer [8], heteroaxin by a fluorometric method [9], vitamin C according to Ivanov [5], vitamin B_1 by the thiochrone method, and sugars and amino acids by the method of partition paper chromatography [10,11].

Germination of grains begins after they absorb 23-28% of water. Here, in all probability, the conditions are created which lead to the formation of substances containing sulfhydryl groups, which activate hydrolysis of reserve proteins. As a result of the latter, free amino acids are formed and physiologically active substances are liberated, which until then had been adsorbed on the proteins [12]. Growth substances entering the swollen embryo cause its growth, and the first visible results of their action are the changes in respiration.

The results of the calculation of respiration rate are given in Table 1.

From the data given in Table 1, it can be seen that a sharp increase in oxygen absorption and decrease in CO_2 evolution is observed in 36 hours. Evidently in the first 24 hours the oxygen does not penetrate into seeds, and the energy for the growth of embryo is obtained by fermentation. As a proof of this, the high values of the respiratory coefficient of seeds prior to swelling may be cited. Sugars are used as a fermentation substrate; these are deposited in the embryo itself. By means of paper chromatography it was possible to find sucrose, glucose, frutose, pentose, and raffinose in the dry rice embryos. Chromatographic analysis of embryos of swollen seeds has shown that the amount of glucose and sucrose in them was decreased, while the amount of fructose, raffinose and pentose did not change quantitatively. After swelling, when the penetration of oxygen into the grain may be accomplished, the reserve starch in the endosperm is thrown into the cycle of biological oxidation.

Increase of respiration rate during germination is the total reflection of the activity of various respiratory enzymes of the seed. Calculation of the action of different terminal oxidases has shown that in rice sprouts which have grown on the moist surface, the cytochrome

TABLE 1. The Respiration Rate of Germinating Rice Seeds (in μliter of gas per 30 min per g of dry weight)

gas exchange	hours of germination			
	12	24	36	48
oxygen absorbed	3.6	8.2	32	48.5
CO_2 evolved	13.7	23.9	18.3	33.95
respiratory coefficient	3.8	2.8	0.6	0.7

and polyphenoloxidase systems are functioning. In addition, with age the role of the residual respiration increases. The results of measurement of enzyme activity and of residual respiration are given in Fig. 1.

This change in respiratory systems takes place during germination of rice seeds on moistened substrate; but what occurs then if the seeds are placed into anaerobic conditions ? In the first condition, as a result of absence of oxygen the oxidases cannot be active. However, in spite of that, the rice-seeds germinate and form coleoptiles (see Table 3). Evidently in such conditions the coleoptiles grow by means of fermentation, and the total dehydrogenases of coleoptiles in the conditions of anaerobiosis considerably exceed those of the sprouts which are placed on a moistened surface. The data on measurement of dehydrogenase activity are given in Fig. 2.

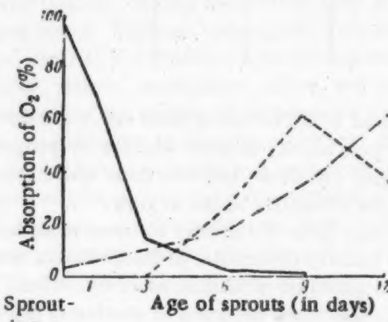

Fig. 1. Activity of oxidase in young rice sprouts. 1) Cytochrome oxidase; 2) polyphenoloxidase; 3) residual respiration.

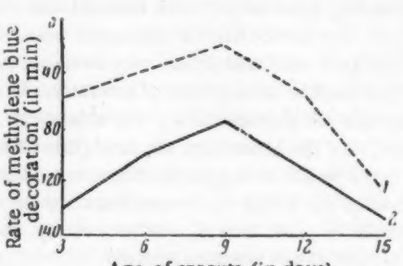

Fig. 2. Activity of dehydrogenases in rice sprouts. 1) Flooding; 2) moistened surface.

Such difference in the action of respiratory systems during moistening and during flooding is a reflection of processes taking place in the germinating seeds. One may judge of these on the basis of measuring pH and Eh of seeds (Fig. 3).

There is a definite relationship between Eh, pH, and the active respiratory system, because Eh is a function of interrelated oxidation-reduction systems and the pH at which a certain reaction takes place. This statement is well emphasized by comparing values of Eh with activity of various respiratory systems. Thus, during moistening, the maximum Eh corresponds to the maximum of cytochrome oxidase activity: When cytochrome oxidase activity is diminished, the values of Eh are also decreased. In anaerobic conditions, the only respiratory systems are dehydrogenases, the oxidation-reduction potential of which is small. Eh in these conditions remains on the lower level and is increased only when sprouts are taken out of the water, when oxidases become active. Similar pictures are observed when Eh and respiratory coefficient are compared.

The respiratory coefficient of germinating seeds at first increases and exceeds unity. Values of Eh at this time are insignificant; after sprouting during moistening and after coleoptile is grown out of the water when flooded, the relationship between evolved CO_2 and absorbed O_2 decreases, but at the same time the values of Eh are increased. The latter phenomenon indicates that the seed germination begins without oxygen reaching

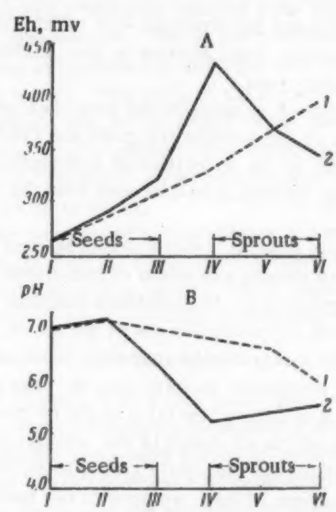

Fig. 3. Change of Eh (Fig. A) and pH (Fig. B) in germinated rice seeds. 1) Flooding; 2) moistening; I) air-dry; II) swollen; III) beginning to sprout; IV) one day old; V) three days old; VI) six days old.

TABLE 2. The Effect of Water Regime on the Absolutely Dry Weight of the Endosperm of Rice Grains (the weight of 100 endosperms in g)

water regime	air-dry seeds	day of germination								
		1	2	3	4	5	6	7	8	9
moistening	2.9	2.9	2.7*	2.5	2.2	1.9	1.7	1.5	1.3	0.9
flooding	2.9	2.9	2.9	2.9	2.8	2.7†	–	–	2.0	1.8

* Sprouting began.

† Coleoptiles grew out of the water.

TABLE 3. Change in Dry Weight of Embryo During Germination and Growth of Coleoptile and Root in Various Media

days of germination	absolutely dry wt. of 100 embryos, g		water, %		coleoptile, cm		root, cm	
	moistening	flooding	moistening	flooding	moistening	flooding	moistening	flooding
first	0.09	0.09	32	33	—	—	—	—
second	0.14	0.08	52	65	0.3	0.7	1.5	0
third	0.21	0.07	65	69	0.7	3.5	4.3	0
fourth	0.34	0.08	66	99	1.0	4.8	5.9	0
fifth	0.48	0.09	67	99	1.7	5.9	6.8	0
sixth	0.84	–	67	–	2.8	7.5	10.0	0
seventh	1.23	–	72	–	–	–	–	–
eighth	1.49	0.15	75	97	–	–	–	–
ninth	1.68	0.28	78	88	–	–	–	–

inside the grain, and the processes with which germination begins acquire great activity in the conditions of prolonged anaerobiosis.

The conditions of anaerobiosis show their effect on the chemistry of the germinating grain, and this is indicated above all by the utilization of endosperm reserve by the growing embryo, as can be seen from the data of Table 2.

In the data given in Table 2 it can be seen that the decrease of reserve substances of endosperm takes place after sprouting on the moistened substrate and after the growth of coleoptiles out of the water, during flooding. This means that in anaerobic conditions the utilization of endosperm reserves does not take place, and the growth of coleoptiles takes place by means of those reserves which are stored in the embryo itself. In such conditions the coleoptile grows, in all probability, by elongation, without synthesis of dry substance. For confirmation of this, measurements were made of the dry weight changes in growing embryo. The results of these measurements were made of the dry weight changes in growing embryo. The results of these measurements are given in Table 3.

It should be noted that in the experiment germination in both water treatments had begun after 36 hours. Seeds which were beginning to germinate were not distinguishable from each other in any way. The differences appeared on the second or third day, when the root began to grow rapidly in seeds which were germinated in moistened condition, while in those which were flooded, the coleoptile began to grow.

One must note that almost 20 times more heteroauxin was found in coleoptiles of flooded seeds than in the sprouts grown on moistened substrate. Thus, 27 μg heteroaxin was found per gram of absolutely dry weight in moistened seeds, while after flooding 569 μg was found.

It should be noted that there is no increase in dry weight in the flooded sprouts, and rapid growth takes place by elongation, as a result of which coleoptiles during flooding grow empty since the subsequent leaves do not grow and do not fill the coleoptile tube. Also coleoptiles grow etiolated, since they were not subjected to illumination of any kind of intensity. In addition, they are not photoperiodic; the absence of photoperiodism, like the absence of accumulation of dry substance, is the result of oxygen insufficiency. The growing of coleoptiles out of the water contributes to the rapid development of green leaves, synthesis of dry substance, and restoration of the reaction to light.

If accumulation of dry weight is compared with the action of the enzyme systems, it can be noted that only in the case of oxidase activity may the processes of formation of dry substance take place. Consequently,

TABLE 4. Dynamics of Vitamin B_1 and Ascorbic Acid in Germinating Rice Seeds

seed condition	vitamin B_1 in μg per g abs. dry weight						ascorbic acid, mg %	
	moistening			flooding				
	free	bound	total	free	bound	total	moist-ening	flooding
air dry	4.00	0	4.00	4.00	0	4.00	0	0
swollen	3.77	0.25	4.02	2.80	1.23	4.03	0	0
sprouted	3.80	1.70	5.50	0.82	3.42	4.24	96	0
germinated	5.40	1.56	6.96	0.00	4.24	4.24	170	0

the oxygen respiration is not only the supplier of "free energy", but also the source of a whole number of intermediate products, without which the synthesis of dry substance is impossible. The latter situation may be illustrated by measurement of free amino acids in coleoptiles when grown in water, as well as in sprouts during their growth on the filter paper.

Using the method of partition paper chromatography, it has been possible to establish that during flooding 17 such amino acids are found: cystine, arginine, lysine, histidine, asparagine, aspartic and glutamic acids, serine, glycine, α-alanine, proline, tyrosine, tryptophane, methionine, valine, phenylalanine, and leucine. During moistening, only 12 amino acids are found: arginine, asparagine, glutamine, glycine, aspartic and glutamic acids, threonine, α-alanine, proline, methionine, valine, and leucine.

Increase of the number of amino acids during flooding is connected in all probability with the break-down in teamwork of physiological processes: Hydrolytic processes do take place, while the synthesis of dry substance does not. In such conditions, there is an accumulation of amino acids in the coleoptile cells.

The absence of the processes of synthesis under anaerobic conditions is also indicated by the dynamics of vitamin B_1 and ascorbic acid (Table 4).

The data of Table 4 show that in dry and in swollen seeds during moistening and flooding ascorbic acid is absent. It appears in nonflooded seeds only after the beginning of germination, while in flooded seeds we have not found it even in three-day-old sprouts.

A certain analogy in the formation of the new substance is observed in relationship to vitamin B_1. In seeds germinated in moist conditions, the transformation of free vitamin B_1 into bound form takes place simultaneously with the synthesis of new amounts of this chemical compound. During flooding only a unilateral transformation of the seed vitamin B_1 into the bound one, into cocarboxylase, is observed. Consequently, during germination of rice seeds in anaerobic conditions the formation of such substances as vitamin B_1 and C does not take place; for their synthesis oxygen is necessary.

If we now sum up all data given, then we may conclude that the germination of rice seeds in non-oxygen conditions permits a more detailed study of those physiological processes which take place in the grain at the very beginning of germination. Up until the beginning of germination they are altogether similar and take place without access of oxygen within the grain, both during moistening and during flooding. Because of this, beginning of germination of grain takes place by sprouting of the coleoptile. Only after this breaking point does the direction of biochemical processes become different during moistening from that which happens during flooding.

In connection with this condition, the germination of rice seeds may be divided into two phases: anaerobic and aerobic. The first lasts from swelling to beginning of germination, and is characterized by the high activity of pyridine dehydrogenases, by the clearly expressed manner of cell elongation and by absence of synthesis of dry substance. The root does not grow until the beginning of germination. The second phase begins with beginning of germination and ends with growth of embryonic leaves. This phase is characterized by dying out of coleoptile and by the growth of the root. Fermentation gives way to oxygen respiration, and then rapid growth of embryonic leaves and synthesis of the dry substance takes place.

The presence of the two phases in growth of rice seeds gives them an opportunity to begin to germinate in a medium lacking oxygen, which explains the high resistance of rice coleoptiles to anaerobic conditions.

SUMMARY

1. Germination of rice seeds takes place in two phases: from swelling up to beginning of germination and from the latter until growth of embryonic leaves. The first phase takes place without entrance of oxygen into the grain, and the beginning of germination is accomplished by the coleoptile without growth of the root. The second phase is characterized by the free access of oxygen into the grain and the rapid growth of the root and embryonic leaves.

2. In the beginning of germination—until sprouting—the main supply of energy for growth of the coleoptile is fermentation. The respiratory coefficient at this time considerably exceeds unity, and the oxidation-reduction potential stays at a low level.

3. The dry substance of the endosperm is not mobilized by the growing embryo until the sprouting of grain, as a result of which the growth of coleoptile is accomplished at the expense of substances which are contained in the embryo itself.

4. After sprouting, when oxygen begins to penetrate inside of the grain, the reserves of the endosperm are drawn into the cycle of biological oxidation; the oxidation-reduction potential grows, which ensures the synthesis of dry substance in growing embryo.

5. The main respiratory systems of embryos up until sprouting are dehydrogenases. After sprouting, to these are added such respiratory enzymes as cytochrome oxidase, polyphenol oxidase, and flavine oxidases.

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EARING OF WINTER PLANTS UNDER CONDITIONS OF RELATIVELY HIGH TEMPERATURE

A. K. Fedorov

Institute of Genetics, Academy of Sciences of the USSR, Moscow
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The question of whether or not winter plants could ear out without preliminary vernalization at low temperatures under conditions of relatively high temperatures has not been resolved until recently, although it has been discussed in the biological literature.

Thus, tests have been made by a number of physiologists to change the natural complex of factors necessary for the stage of vernalization by the action of continuous illumination [1,2].

The authors [1, 2] obtained ear development in their experiments with several varieties of winter grains by sowing nonvernalized seeds under conditions of continuous illumination (additional lighting from ordinary incandescent lamps).

However, similar work was criticized on several counts: an incorrect approach to the studied effect, imprecision of the method, especially in the idea of maintaining constant temperature conditions in different variants of the experiment, and inconclusive final results. For example, Avakyan and Tagi-Zade [3] carried out investigations to check the ideas put forth by M. Kh. Chailakhyan. As a result, they came to the conclusion that in the latter's experiments on the open ground, vernalization took place under the effect of a relatively decreased temperature, from which one is able to explain the earing out of winter plants in his experiment. Earing of winter plants under conditions of continuous illumination was not observed in the experiment of Avakyan and Tagi-Zade [3], in which the plants were not subjected to the action of a decreased temperature. We must note parenthetically that in our experiments, winter wheat sowed with nonvernalized seeds in the spring in the open ground and in the greenhouse, both under conditions of continuous illumination (additional lighting from 500-watt incandescent lamps) and also under conditions of natural illumination, up to the end of the vegetation period did not ear out. The same result was also observed in an experiment with perennial winter grasses (meadow fescue, orchard grass, white bent grass, and others).

Analysis of the data that we obtained on the study of the nature of alternative plants, especially the first generation hybrids from crossing summer wheat and the alternative with winter wheats, led us to conclude that winter plants can ear out at relatively high temperaures after being subjected to the previous effect of low temperatures.

The work that we carried out [4,5] with a relatively large number of varieties of alternatives of different agricultural crops (wheat, barley, oats, vetch, and perennial grasses) from various climatic regions showed that a retardation in development and growth significantly greater than for summer plants takes place under the effect of autumn conditions. This characteristic has an adaptive significance and is strongly expressed for winter resistant plants. The alternatives that were subjected to the continuing action of low temperatures (30-50 days) are retarded significantly less in development under short-day conditions.

We carried out crosses of summer varieties, alternatives and winter varieties, followed by a study of the first generation hybrids, in order to deepen our knowledge of the nature of alternative plants, and also to explain the nature and differences of alternatives from summer and winter plants.

Sowing was carried out on different dates under various light and temperature conditions.

Such work was carried out over a period of several years. We are presenting the data for one of the experiments as an example (Table 1).

The data in Table 1 show that with sowing in the spring for first generation hybrids resulting from crosses of summer wheats with alternatives and summer wheats with winter wheats, the point of growth is differentiated (on the eared tubercle) and earing out is observed at approximately the same time for related summer varieties.

Development for hybrid plants of these combinations sowed in the autumn (August 11 and 21) is retarded significantly in comparison to the natural related summer varieties. Thus, for example, differentiation of the point of growth was retarded 30 days for hybrid plants from a cross of Lyutestsens 329 winter wheat with Lyutestsens 62 summer wheat sowed on August 11, as

TABLE 1. The Effect of Dates of Sowing on the Development of First Generation Hybrids

object	date of differentiation of the point of growth	duration of period (in days) from shoots to differentiation of the point of growth	date of earing out
sowed April 26			
F_1 26191 (Alternative) × Lyutestsens 62 (summer)	May 25	15	June 30
F_1 Kooperatorka (winter) × Lyutestsens 62	May 25	15	July 1
F_1 Lyutestsens 329 × Lyutestsens 62	May 26	16	July 3
F_1 Lyutestsens 329 × Moskovka	May 27	17	July 5
F_1 26191 X Kooperatorka	June 27	48	Aug. 5
F_1 Kooperatorka × 26191	June 30	51	Aug. 8
F_1 Moskovskaya 2453 × 26191	July 14	64	Sept. 3
F_1 Lyutestsens 329 × 26191	July 19	69	Sept. 9
Lyutestsens 62 (summer)	May 23	13	June 28
26191 (alternative)	June 3	24	July 10
Lyutestsens 329 (winter)	Sept. 14	127	none
sowed August 11			
F_1 26191 × Lyutestsens 62	Sept. 25	36	none
F_1 Kooperatorka × Lyutestsens 62	Sept. 25	36	the same
F_1 Lyutestsens 329 × Lyutestsens 62	Oct. 8	49	"
F_1 Tule II (Swedish) × Lyutestsens 62	Oct. 9	50	"
F_1 Kooperatorka × 26191	none	–	"
F_1 Lyutestsens 329 × 26191	the same	–	"
Lyutestsens 62	Sept. 8	19	"
26191	Oct. 25	66	"
109 (alternative)	Oct. 7	48	"
sowed August 21			
F_1 26191 × Lyutestsens 62	Oct. 16	49	none
F_1 Kooperatorka × Lyutestsens 62	Oct. 22	55	the same
F_1 Lyutestsens 329 × Lyutestsens 62	none	–	"
F_1 Tule II (Swedish) × Lyutestsens 62	the same	–	"
F_1 Kooperatorka × 26191	"	–	"
F_1 Lyutestsens 329 × 26191	"		"
Lyutestsens 62	Sept. 24	27	"
26191	none		"

compared to Lyutestsens 62 summer wheat. Based on the degree of this retardation, hybrid plants are similar to wheat alternative 109 (a variety from Bulgaria).

A retardation not only in the development, but also in the growth is observed for hybrid plants sowed in the autumn. The capacity for development and growth is sharply retarded under the effect of autumn conditions, and this is a characteristic feature of alternative plants.

The data in Table 1 further show that hybrid plants of the first generation cross of the alternative with winter wheat and on spring sowing are significantly retarded in growth, exist more than half of the summer in the phase of stem branching, and resemble winter plants in their external appearance. However, in comparison to the latter, hybrid plants simultaneously ear out in August and the beginning of September (in relation to related varieties). Hybrid plants ear out earlier in combinations where Kooperatorka was taken as a winter variety and later where Lyutestsens 329 winter wheat was taken. This is an extremely interesting fact, one which we must ponder. With autumn sowing dates, first generation plants of crosses of alternatives with winter wheat varieties, located in the phase of stem formation with a nondifferentiated point of growth, are similar in external appearance to the usual winter wheat varieties.

Experiments on the study of the effect of vernalization of seeds on the development of the first generation of hybrid plants were carried out. It appeared that

TABLE 2. The Effect of the Length of Day and Vernalization of Seeds on the Development of First Generation Hybrids from Crosses of Kooperatorka Wheat with Alternative 26191

length of day	types of seeds sowed	date of differentiation of the point of growth	lag for nonvernalized seeds compared to vernalized seeds (days)	date of earing out
natural	nonvernalized	June 30	33	Aug 8
	vernalized	May 28	–	July 2
short 12-hour day	nonvernalized	July 31	42	none
	vernalized	June 19	–	Aug 15

the first generation of hybrids after grafting summer wheat with winter wheat on spring and summer sowing hardly accelerates in development under the effect of vernalization of the seeds. With sowing in the autumn or with spring and summer sowing under short-day conditions, a significant reaction is observed on vernalization. Thus, in one of the experiments under conditions of a twelve-hour day, the difference in the rates of development of hybrid plants sowed with vernalized and nonvernalized seeds was 34 days.

The short day retards significantly the development of first generation plants in comparison to plants of the related summer variety. Thus, with sowing on April 26, plants of Lyutestsens 62 summer wheat eared out 35 days later under short day conditions than under natural conditions, but hybrid plants of the first generation after crossing Lyutestsens 329 winter wheat with Lyutestsens 62 did 56 days later.

Now we will discuss the data obtained in the study of the effect of vernalization and different conditions of illumination on hybrid plants of the first generation after crossing alternatives with winter wheat.

Development of hybrid plants of the given combination of vernalization of seeds was significantly accelerated not only in the short day conditions, but also in the natural day conditions of a relatively long day. As a special experiment carried out according to the usual method showed, approximately 45-50 days are required to vernalize hybrid seeds. There is nothing remarkable in this,because the same number of days is required for vernalization of the related winter variety of Lyutestsens 329. Practically the same number of days is required to vernalize seeds for the other related variety alternative 26191 in order to obtain a significant acceleration in development in short-day conditions. We will discuss the data of the experiment in which the effect of vernalization of seeds under various lengths of day was studied.

The data of Table 2 show that hybrid plants from vernalized seeds develop significantly more rapidly than plants from nonvernalized seeds under natural day conditions, which was not observed for hybrid plants from crosses of summer wheat with alternatives and winter wheat. Under short day conditions, the difference in the rate of development between hybrid plants from vernalized and nonvernalized seeds increases still more. Further, the short day retards the development of plants from nonvernalized seeds.

In connection with this, it was interesting to explain the effect of continuous illumination on the development of hybrids. With this as a goal, sowing of hybrids of this same combination (Kooperatorka × 26191) was carried out on April 29 with nonvernalized seeds on a light plot; after complete sprouting by the plants, various conditions of illumination were applied; natural, continuous (additional illumination from 500 watt incandescent lamps with intensive lighting of about 6000 lux) and short-day (12 hours). The results of the experiment in Table 3.

The data in Table 3 show that the conditions of illumination significantly affect the development of first generation plants. Continuous illumination accelerates the development of plants in comparison to natural illumination, but the short day significantly retards their development.

With this, as our experiments showed, not only the length of the day but also the intensity of the illumination significantly affect the rate of development of plants of the first generation crosses of Lyutestsens 329 winter wheat with alternative 26191. Thus, with continuous illumination at an intensity close to 70,000 lux, F_1 eared out 41 days after complete sprouting (Lyutestsens 62 summer wheat after 27 days; F_1 crosses of Lyutestsens 329 × Lyutestsens 62 after 28 days), and with a light intensity of about 4500 lux, only after 75 days (Lyutestsens 62 summer wheat after 45 days; F_1 crosses of Lyutestsens 329 with Lyutestsens 62 after 46 days).

In general, if the three combinations of first generation hybrids are compared, then we must note that

TABLE 3. The Effect of Conditions of Illumination on the Development of First Generation Hybrids from Crosses of Kooperatorka Winter Wheat with Alternative 26191

conditions of illumination	date of differentiation of the point of growth	duration of period from full sprouting to differentiation of the point of growth	date of earing out
natural	July 10	56	Aug 12
continuous	June 10	26	July 10
short day	Aug 14	91	none

the vernalization of seeds hardly affects the rates of development of plants from crosses of summer wheat with the alternative and winter wheat under relatively long day conditions and significantly accelerates their development under short day conditions. On plants of the first generation crosses of alternative wheat with winter wheat, vernalization of the seeds is suggested not only under short day conditions, but also under relatively long day (natural summer) conditions.

With sowing in the spring and first half of the summer, that is, under relatively long day conditions, hybrid plants of the first generation crosses of summer wheat with alternative,and summer wheat with winter wheat, hardly differ from the normal summer varieties in rates of development. The first generation of hybrids of crosses of alternative wheat with winter wheat in these same conditions is significantly retarded in development, approaching winter wheat in this.

Under short day conditions (with autumn sowing dates or in the spring, but with artificial shortening of the natural day), first generation plants of all three combinations are significantly retarded in cevelopment in comparison to the usual summer varieties (Lyutestsens 62, Moskovka). Less retardation in development under short day conditions is observed for first generation crosses of summer wheats with alternatives and the most for hybrids from crosses of alternative wheats with winter wheats.*

Thus, it appears that first generation hybrid plants of one combination differ from the other according to the reaction in light conditions during the beginning period of life (with sowing of nonvernalized seeds, prior to the continuing action on them of a low temperature). If this is so, then there is a basis to assume that natural varieties related to various types of development—summer, alternative, and winter wheats—probably must also differ in the reaction to light conditions during the beginning period of their life (with sowing of nonvernalized seeds prior to the continuing action of a low temperature on them).

We saw above that actually summer and alternative wheats differ in their reaction to light conditions, particularly in retardation in development in short day conditions and relatively weak illumination. Thus, summer, alternatives, the first generation of hybrid crosses of summer with alternatives, summer with winter, and alternatives with winter differ in their reactions to light conditions. Some of them are retarded under short-day conditions; others are also retarded under long-day conditions. They also react differently to the light intensity.

Because summer, alternative and hybrid plants from their crosses, especially hybrid plants from crossing summer with winter and alternatives with winter, differ in their reactions to light conditions, there is reason to suppose that winter plants must differ also in development.

If all that has been said is presented for demonstration in a rough form, generally approaching a scheme (Fig. 1), then the following results are obtained.

If the duration of the period from complete sprouting up to differentiation of the point of growth (or up to earing out) is represented on the vertical axis, and forms differing in the type of development are represented on the horizontal axis: (1) summer, (2) F_1 cross of summer alternative, (3) F_1 cross of summer with winter, (4) summer with alternative,(5) F_1 alternative with winter, (6) winter—then a rising curve is obtained (along the length of the period up to earing out) from the summer to the winter side. Under short day conditions the slope of this curve is significantly greater (Fig. 1, I) than under conditions of continuous intensity of lighting (Fig. 1, II). With sowing of vernalized seeds under long-day conditions or continuous and normal temperature (15-20°), the difference in the reaction to light for different groups of plants to a significant degree is smoothed out; this is shown in Fig. 1, III. From this we see that of the six groups of plants differ-

* Undoubtedly, this would depend not only on the type of development possible for the crossing of the variety, but also on the features of the varieties, because there are differences in the nature of the variety among plants of other types of development.

ing in types of development, for five their differences are stipulated to a great degree by the different reaction to light. All of this broken up material inevitably leads us to the fact that the sixth group of plants, the winter plants, also must differ from the remaining five by their reaction in light. The entire analysis of the material obtained, especially based on the study of the biology of the first generation of hybrids from crossing alternatives with winter plants, led us to conclude that winter plants must ear out on fairly intensive continuous lighting without their preliminary vernalization at low temperatures. For an indication of this, a special illuminating instrument with a water filter and 500-watt reflector lamps was constructed.

Winter wheat and rye were sowed in planting jars and were transferred to intensive artificial illumination of about 50-70 thousand lux after complete sprouting, and also to other light conditions. The temperature in the experiment did not fall below 20°.

Two series of experiments were carried out. In the first series, sowing was carried out on April 4, in the greenhouse; on April 11 after complete sprouting, the containers with the plants were placed in various conditions: in continuous intensive artificial lighting (about 50-70 thousand lux); in continuous lighting where ordinary incandescent lamps with an intensity of about 5000 lux were used as sources of additional lighting; and under natural conditions. On June 20, that is, 70 days after complete sprouting, a massive earing out was noted in the first variant for Bankuti 1201 winter wheat, and on July 7 for Vyatka Moskovskaya winter rye, that

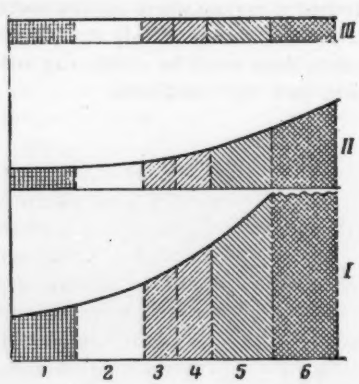

Fig. 1. Approximate scheme of the reaction of plants of various types of development to light conditions. I) Sowing nonvernalized seeds under short-day conditions; II) sowing nonvernalized seeds under long-day conditions with intensive lighting; III) sowing vernalized seeds under long-day conditions; 1-6) explanation in text.

is, after 87 days. Later a massive earing out was observed for winter wheat Moskovskaya 2453 and Lyutestsens 329 on August 18, that is 129 days after complete sprouting. At the same time in the other two variants, winter plants up to the end of the vegetation period did not ear out, remaining in the phase of stem branching. (Actually, the beginning of emergence in tube for a part of the plants of Bankuti 1201 winter wheat was noted in the second half of September.)

Thus, the proposition derived from an analysis of experiments with first generation hybrid plants that winter plants must ear out under conditions of intensive, continuous lighting without preliminary vernalization was supported. This is shown in Fig. 1, II. However, for greater conculsiveness, we established another similar experiment.

Sowing was carried out on September 14 in planting containers of Vyatka Moskovskaya winter rye.and winter wheats Bankuti 1201, Kooperatorka, Moskovskaya 2453 and Lyutestsens 329, alternative wheat 26191, Lyutestsens 62 summer wheat, F_1 from a cross of Lyutestsens 329 winter wheat with alternative 26191. After the plants had completely sprouted, they were placed under intensive artificial continuous lighting (about 50-70 thousand lux) on an instrument, on continuous lighting (natural + additional from ordinary 500 watt incandescent lamps with intensive lighting of about 5000 lux), and on natural lighting.

As a result, winter plants under conditions of intensive continuous illumination eared out together after 70-105 days (Fig. 2). Under conditions of relatively weak illumination, plants remained in the phase of stem branching †, but on the natural day they died from light deficiency.

The duration of the period from complete sprouting to earing out for different varieties under conditions of continuous intensive illumination is seen from the data in the table on page 572.

The data that are presented show that, as in the first experiment, winter varieties eared out (Fig. 2). A winter variety with a less strongly expressed winter characteristic, Bankuti 1201, eared out earlier than winter varieties Moskovskaya 2453 and Lyutestsens 329.

Winter varieties with a less strongly expressed winter character ear out under conditions of intensive illumination at a high temperature more rapidly than varieties with a more strongly expressed winter character. The most rapid earing out was observed for the summer variety; alternative wheat eared out later than

† It is interesting to note that winter plants contained much more sugar in their leaves under intensive illumination than under the ordinary continuous lighting. Thus, for Moskovskaya 2453 winter wheat on intensive, continuous lighting, the leaves contained 17.4% sugar based on dry weight, and on ordinary continuous lighting (5000 lux) they contained only 7.48%.

the summer variety and significantly earlier than the winter varieties. Thus, a rising curve is obtained with respect to the length of the period from complete sprouting to earing out, from summer plants through alternatives to winter plants. We must note that the differences observed for varieties based on the length of the indicated period in general fall in the period from complete sprouting up to differentiation of the point of

variety and combination	length of period from complete sprouting to earing out (in days)
winter wheats and rye	
Lyutestsens 329	105
Moskovskaya 2453	105
Kooperatorka	97
Bankuti 1201	81
Vyatka Moskovskaya winter rye	70
F_1 Lyutestsens 329 × 26191	52
alternative wheat 26191	43
summer wheat Lyutestsens 62	37

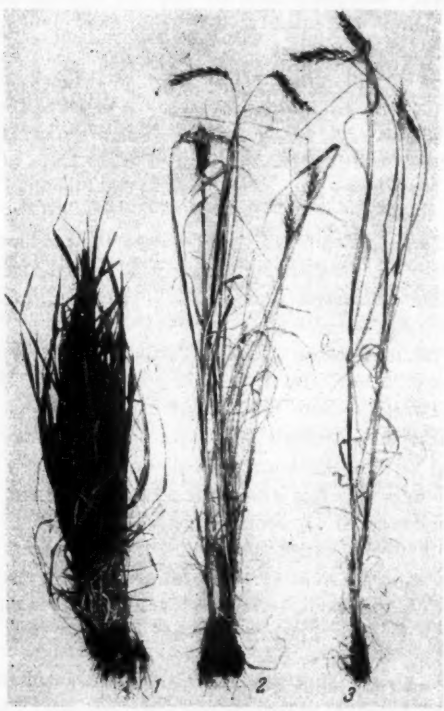

Fig. 2. Winter wheat plants. 1) Continuous illumination with an intensity of about 5000 lux; remained in the stem branching phase; 2) Moskovskaya 2453; 3) Lyutestsens 329 in continuous illumination with an intensity of 50-70 thousand lux, eared out.

growth. Probably one can assume that the shift of the point of growth from a vegetative condition to a generative condition is possible for summer varieties after the action by a smaller amount of light energy than for alternatives, and for alternatives less than for winter plants. Winter varieties in the other variants did not ear out.

Under conditions of intensive (50-70 thousand lux and more) continuous illumination in our experiments at relatively high temperatures, perennial winter grasses also eared out: meadow fescue (Fig. 3), orchard grass, white red top, meadow foxtail, and smooth brome. The same picture as for the grains was also observed here. Grass alternatives eared out earlier than the winter grasses. Winter perennial grasses of varieties with a less strongly expressed winter character eared out earlier than varieties with a more strongly expressed winter character. Thus, for example, meadow fescue of southern origin (from Kirghiz) K - 1852a, eared out (with establishing it in the stem branching phase on intensive lighting) after 25 days, and meadow fescue of norther origin (Khibinskaya 806, K-2489) only after 44 days. All of this shows that one of the differences of winter plants from other types is the different reaction to the light conditions during the beginning period of life (up to the end of the vernalization stage).

In conclusion it must be noted that for winter plants in the process of their historical development, an adaptive characteristic was probably developed to retard the plant in development in light conditions preceding winter misfortune.

For alternative plants, the cultivation of which most often is limited to regions where autumn sowing is carried out fairly late, with relatively short days and weak light intensity, there would be a short day and weak light intensity with such light conditions.

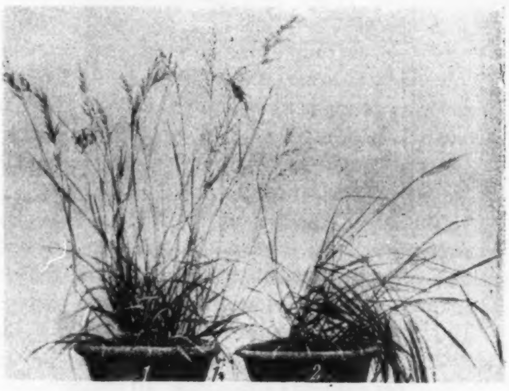

Fig. 3. Meadow fescue plants. Left—continuous illumination with an intensity of 50-70 thousand or more lux eared out; right—continuous illumination with an intensity of about 5000 lux remained in the stem branching phase.

For winter plants, which are most often cultivated in regions where autumn sowing of grains is done with a long day and fairly intensive illumination, such a light condition would be a relatively long day and fairly intensive illumination.

In light conditions exceeding the ordinary natural illumination in their level of intensity of autumn sowed plants, earing out is possible without the preliminary action of decreased temperatures. For alternative plants it would take natural conditions (average northern region) for spring-summer, but for winter plants, it would take conditions similar to those under which winter wheat was cultivated in our experiment (continuous illumination with an intensity of about 50-70 thousand lux).

SUMMARY

One of the differences of summer plants, alternatives, and winter plants is the different reaction to light conditions.

After the continued action of decreased temperatures there takes place a change in the reaction to light conditions, and differences in the reaction of plants differing in type of development to a significant degree are smoothed out (for example, by sowing vernalized seeds).

This makes it possible to approach an explanation of a number of insufficiently clear facts in biological literature connected with the nature of winter plants, alternatives, perennial grasses, with the propagation of fairly long day plants in Abyssinia where the natural length of the day does not exceed 12 hours, 40 minutes, etc. This makes it possible to approach more closely an understanding of the role of the light factor with the managed change of plants; in particular it becomes clearly why with later autumn sowing of summer wheat its descendents are usually more of the alternative type, but with relatively early sowing dates, an opposite impression is observed to one degree or another.

All of this shows that one of the reasons for the different length of the vegetation period, type and rates of development, and differences in resistance to unfavorable conditions, is the dissimilar reaction of plants to light conditions, especially in the initial period of life.

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SEVERAL PHYSIOLOGICAL FEATURES OF HYBRIDS AND INITIAL SELF-POLLINATED LINES OF CORN

M. S. Rubtsova

Gor'kii Agricultural Institute

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The phenomenon of heterosis is widely used in the practice of agriculture to obtain high-yielding hybrids of corn; therefore, knowledge of the physiological features of this phenomenon is necessary.

The basic means of discovering the physiological nature of heterosis lies in a comparison of the physiological and biochemical features of hybrids and initial self-pollinated lines. While sharply differing in their anatomical and physiological features [1-12], hybrids and self-pollinated lines undoubtedly have still deeper differences in the intensity and character of the physiological processes.

To discover these differences, a number of authors carried out comparisons of the intensity of respiration [13,14], photosynthesis [13-15], rate of translocation of substances marked with C^{14} [15], the activity of the oxidizing enzymes [16], the quantity of growth substances [12] and several other indicators, for hybrids and self-pollinated lines.

However, such work carried out up to the present time is still scanty, and the data obtained by different authors have a rather contradictory character.

On the basis of the data in the literature and our own observations, the idea was suggested that the physiological-biochemical differences in the flow of the metabolic prccesses in hybrids and self-pollinated lines to a known degree are connected with differences in their intercellular oxidation-reduction regime [16].

This article, which represents the results of three years of work, has as its objective the presentation of additional facts in this direction.

METHODS

Double interline hybrids of corn VIR 25, and VIR 156 with their initial simple interline hybrids VIR 28 × 29, 26 × 27, 40 × 43, 44 × 38, 157 × 158, 64 × 133, and the corresponding self-pollinated lines 28, 29, 26, 27, 40, 43, 44, 38, 157, 158, 64, and 133 served as experimental plants. Plants of hybrids and lines were grown under similar conditions in fields of the Educational-Research Farms "Shcherbinki" and "Novinki" of GSKI. There were three plot repetitions. Mineral and organic fertilizers were applied in the holes at sowing time. Sowing was carried out in square depressions, with holes spaced 70×70 cm.

Leaves and stems of the plants were taken for analysis. We measured the intensity of photosynthesis and respiration by the method of Pochinok [17]; the productivity of photosynthesis according to Nichiporovich [18]; the activity of the oxidizing enxymes according to Povolotska and Sedenko [19]; the oxidation-reduction potential in the flow of nitrogen and pH by electrometrical methods [20]; total and protein nitrogen by the micro-Kjeldahl method; the concentration of free amino acids and amono acids attached to the protein by the distributed paper chromotography method.

DATA AND DISCUSSION

Photosynthesis and respiration are the basic processes determining the oxidation-reduction tone of the leaf cells. A comparative study of photosynthesis for hybrids and self-pollinated lines of corn showed that hybrids are characterized by greater intensity of this process than self pollinated lines. Some of the obtained data are presented in Fig. 1, A. An inverse rule was observed by us with respect to respiration. The intensity of respiration, as we can see in Fig. 1 B, is significantly higher for self-pollinated lines than for hybrids.

This relationship of photosynthesis and respiration indicates that hybrids are more productively in photosynthesis than the self-pollinated lines (Table 1).

The increased intensity of respiration of the self-pollinated lines of corn made it possible to assume a higher activity of oxidizing enzymes in the leaves of these plants.

The analyses carried out show that such oxidizing enzymes as ascorbic acid oxidase, polyphenol oxidase, and peroxidase are significantly more active in the leaves of self-pollinated lines than in the hybrids (Tables 2 and 3).

The high intensity of respiration and increased activity of the oxidizing enzymes show that in the leaf cells of self-pollinated lines a more oxidized environment exists. The oxidation-reduction potential (Eh) can serve to a significant degree as a total indicator of the oxidation-reduction conditions of the intracellular environment.

TABLE 1. Increase in Dry Substance of Corn Plants in One Day, by Periods (in gram) 1959 Experiment

Periods	VIR 25		VIR 28×29		VIR 29	
	per plant	per m^2 of leaves	per plant	per m^2 of leaves	per plant	per m^2 of leaves
July 30–August 10	5.00	12.61	2.86	8.45	2.24	8.15
August 10-August 17	3.31	6.61	4.44	10.93	0.68	1.09
August 17-August 24	1.70	3.30	3.32	7.37	1.95	2.30
August 24-September 8	2.96	4.30	1.63	3.52	0.55	1.07

TABLE 2. Activity of Ascorbic Acid Oxidase and Peroxidase,* Oxidation-Reduction Potential, and pH of Leaves of Interline Hybrids of Corn VIR 157×158, VIR 133×64, and Initial Parent Forms (1958 experiment)

Hybrids and self-pollinated lines	Tube formation				Tassel formation				Tassel flowering			
	ascorbic acid oxidase	per-oxidase	Eh, volts	pH	ascorbic acid oxidase	per-oxidase	Eh, volts	pH	ascorbic acid oxidase	per-oxidase	Eh, volts	pH
VIR 157×158	196.0	6139.0	0.4036	5.67	163.1	4730.2	0.4122	5.58	73.1	3128.4	0.4346	5.63
VIR 157	225.8	7291.7	0.4646	5.56	178.4	5124.0	0.4387	5.48	132.7	3888.2	0.4387	5.31
VIR 158	226.0	6887.9	0.4412	5.24	176.5	4920.2	0.4770	5.46	122.3	3473.6	0.4770	5.58
VIR 133×64	199.0	7070.5	0.3942	5.63	154.0	4706.8	0.3820	5.45	71.3	2445.2	0.4387	5.76
VIR 133	249.5	7348.7	0.4634	5.47	164.2	4612.6	0.4309	5.44	75.0	2841.2	0.4425	5.76
VIR 64	218.5	7185.6	0.4617	5.31	183.4	5283.4	0.3820	5.49	100.7	3471.5	0.4710	5.58

* Activity of ascorbic acid oxidase and peroxidase expressed in mg of ascorbic acid oxidized by the enzymes in 30 minutes per gram of dry weight of tissue.

It follows from Table 2 that the Eh in the leaves of the self-pollinated lines shifts to the side of oxidation, but the pH lies in a more acid range than for hybrids.

Additionally, we carried out measurements of the activity of the oxidizing enzymes in the plant stems. It was established that the stems of self-pollinated lines of corn, just as the leaves, have a higher activity of oxidizing enzymes. Only during the early phases of development of plants is a somewhat different picture observed. Some of the data that were obtained are presented in Table 4.

Thus, tissues of the aerial organs of self-pollinated lines apparently are characterized by a less reduced intracellular medium than tissues of hybrids.

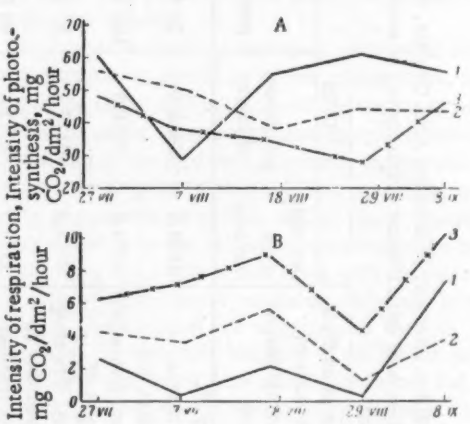

Fig. 1. The intensity of "actual" photosynthesis (A) and respiration (B) of hybrids and self-pollinated lines of corn. Midday figures; 1959 experiment. 1) Double interline hybrid VIR 25; 2) simple interline hybrid VIR 28×29; 3) self-pollinated line VIR 29.

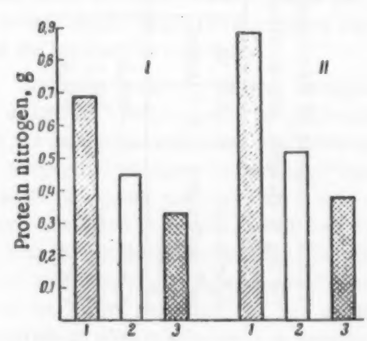

Fig. 2. The concentration of protein nitrogen in the leaf parenchyma of hybrids and self-pollinated lines of corn (in g per plant). 1959 experiment. I) Tube formation phase; II) phase of tassel flowering; 1) Double interline hybrid VIR 42; 2) simple interline hybrid VIR 44 x 38; 3) self-pollinated line VIR 44.

TABLE 3. Activity of Polyphenol Oxidase in the Leaves of Hybrids and Self-Pollinated Lines of Corn (in mg of ascorbic acid oxidized by the enzyme in 30 min per 1 g dry wt.of tissue: 1959 experiment)

hybrids and self-pollinated lines	July 13, 6-7 leaves	July 28, 10-11 leaves	August 20, emergence of the tassel	August 25, flowering of the tassel and ears	Sept. 2, milk ripeness	Sept. 19, milk-wax ripeness
VIR 25	466.2	1256.0	3913.6	3824.1	1695.4	2380.1
VIR 26×27	396.1	1257.3	3768.6	3834.2	3216.9	2231.8
VIR 26	504.4	1382.7	4105.6	4514.5	2648.8	2626.1

TABLE 4. Activity of Ascorbic Acid Oxidase, Polyphenol Oxidase, and Peroxidase in the Stems of Double Interline Hybrid of Corn and Several Initial Parent Forms (in mg of ascorbic acid oxidized by enzyme in 30 min per g of dry weight of tissue; 1959 experiment)

date of analysis and phase of development of plants	VIR 25			VIR 26 × 27			VIR 26		
	ascorbic acid oxidase	polyphenol oxidase	peroxidase	ascorbic acid oxidase	polyphenol oxidase	peroxidase	ascorbic acid oxidase	polyphenol oxidase	peroxidase
July 28, 10 leaves	1694.9	2445.9	29 413.2	1388.7	3253.7	28 952.1	1389.7	2604.6	25 204.1
August 20, tassel formation	4974.8	7018.8	168 847.6	5480.6	7208.7	170 901.4	5722.7	7222.9	184 175.4
August 25, tassel flowering	3072.2	6176.2	51 790.3	3212.0	6969.2	516 774.4	4215.0	7948.2	99 233.9
September 2, milk ripeness	2237.7	3771.4	92 675.4	–	4660.5	101 004.8	2286.8	5777.1	117 738.6

TABLE 5. Content of Protein and Nonprotein Nitrogen in the Leaves of Hybrids and Self-Pollinated Lines of Corn (percent of weight of air-dried tissue; 1959 experiment)

Hybrids and self-pollinated lines	Tube formation		Tassel flowering	
	protein nitrogen	nonprotein nitrogen	protein nitrogen	nonprotein nitrogen
VIR 42	3.46	0.14	2.75	0.17
VIR 40×43	3.47	0.11	2.65	0.14
VIR 44×38	3.65	0.11	2.62	0.09
VIR 40	3.47	0.16	2.46	0.32
VIR 43	3.37	0.30	2.84	0.25
VIR 44	3.53	0.35	2.69	0.20
VIR 38	3.27	0.29	1.93	0.25

According to several authors [21,22], the shift in the intracellular oxidation-reduction regime to the side of oxidation negatively affects the synthesis of a number of important substances, including the proteins. Therefore, we considered it necessary to compare the concentration of protein nitrogen of hybrids and self-pollinated lines per unit of dry weight and for all leaves of a single plant. The data are presented in Table 5 and Fig. 2. From the data that are presented it follows that although in many cases the self-pollinated lines are characterized by a lower concentration of protein nitrogen per unit of leaf weight than hybrids, this difference is very insignificant. Differences are not observed in the amino acid composition of the proteins of both groups of plants. Apparently the lines possess practically the same ability for the synthesis of protein as the hybrids. Also the total accumulation of protein nitrogen by the leaves of a single plant is somewhat lower for the self-pollinated lines than for the hybrids (Fig. 2). The difference in the total concentration of protein is connected with the fact that the self-pollinated lines have a significantly lower rate of growth of vegetative mass than the hybrids.

For this same reason an increased quantity of nonprotein nitrogen, in particular of free amino acids, is accumulated in the leaf cells of self-pollinated lines, as the results of the chromatographic analysis showed. We know that in the processes of growth, the growth substances are of great importance, and according to the data of a number of authors [23,24] these substances are very sensitive to the oxidizing condition of the cell medium. They are easily broken down with the presence in the cells of highly active oxidizing enzymes. In this connection, the results of our experiments lead to the idea that the increased intensity of the growth processes for self-pollinated lines can be connected not so much with the disruption of biological syntheses as with the retarding of growth processes as a result of the oxidation of several groups of growth substances.

This idea corresponds to the results of the experiments by Matskov and Manzyuk [12], who discovered that the self-pollinated lines of corn are characterized by systems deficient in growth substances. It is possible that this is connected with the breakdown of part of the growth substances in the leaves and stems of self-pollinated lines by the more actively oxidizing enzymes.

Thus, the experimental material presented in the article shows that heterosis hybrids of corn differ from their inbred parents by the fact that they apparently have a more reduced intracellular environment. This can create more favorable conditions for the realization of the growth processes.

SUMMARY

1. Corn hybrids have a higher intensity of photosynthesis and decreased respiration than self-pollinated lines. A relationship more favorable to the accumulation of organic substances between photosynthesis and respiration for hybrids leads to an increase in the productivity of their photosynthesis.

2. The leaves and stems of corn hybrids, in contrast to the analogous organs of self-pollinated lines, are characterized by lower activity of the oxidizing enzymes, lower levels of Eh, and shifts in the pH to a lower, acidic range, which raise in them the reduction level of the intracellular medium.

3. The concentration of protein nitrogen per unit of leaf weight for hybrids is only insignificantly higher than for the self-pollinated lines, and sometimes is lower. Nonprotein nitrogen, in particular that of free amino acids, is usually greater in the leaves of self-pollinated lines than in hybrids, which could be connected with the weakened use of it in the formation of protein in the young organs as a result of decreased intensity of the growth processes. To sum up, the total concentration of protein substances is significantly higher for hybrids than for self-pollinated lines.

4. It is suggested that the higher reduction level within the cells of hybrids rather than promoting the synthesis of protein creates more favorable conditions for the formation and action of the growth processes.

I extend deep thanks to Professor V. P. Nogtev for directing this work.

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THE EFFECT OF EXTERNAL CONDITIONS ON THE MONO- AND OLIGOSACCHARIDE CONTENT OF WHEAT LEAVES

O. A. Korchagina

K. A. Timiryazev Institute of Plant Physiology, Academy of Sciences of the USSR, Moscow

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Long ago many researchers turned their attention to the climatic conditions in which plants grow in the extreme North. A short summer, a low sun angle and a relatively high intensity of illumination in the night hours of the summer months are characteristics of Arctic areas.

Our objective was the study of the nature of the carbohydrate metabolism of crop plants grown in the Zapolyar'e (Arctic area). In 1955 comparative-geographical experiments were established with wheat on the Kola peninsula (experimental field of the Kola section of the USSR Academy of Sciences in the region of Apatity, 67° 37' north) and in the region of Moscow (experimental plot of the Institute of Plant Physiology, USSR Academy of Sciences, in the Lenin mountains, 55° 40' north).

Two varieties of summer wheat, Lyutestsens 62 and Khibiny 314, were sowed in the field; each experimental variant had eight plots, each 10 square meters, sown with the normal amount of seed. A complete dose of fertilizer was applied immediately prior to sowing. As soon as a majority of the sprouts had appeared, plants in Apatity were divided into two groups: On four plots the plants remained entirely on the natural day, while on the other four plots the plants were given a 12-hour day, cutting off light with photoperiodic chambers from 6 P.M. to 6 A.M.

Several indications of the phenology are presented in Table 1. They show that the time from sowing to the appearance of sprouts was seven to eight days both in the Zapolyar'e and in the Moscow experiments. Samples during the period of stem branching were taken when the plants of the Moscow experiment were 19-20 days old (counting from the appearance of shoots) and when the northern variants were 28-29 days old, because for the latter, especially in the short-day variant, the development of plants in the beginning period, as in the following period, took place slowly due to the somewhat decreased temperatures: The average daily temperature for the period of sprouting to flowering in Apatity was 3.3° lower than in Moscow.

The length of the period from the time when the samples were taken in the phase of stem branching until the beginning of flowering was 28 days for plants in the Moscow experiment and 40 days for plants in the North; in spite of hot days at the beginning of August, the air temperature reached 28° at a height of 20 cm from ground level, and it reached 30° and more at ground level on several days.

Samples of plants were taken six times over the period of a day at four-hour intervals both in the phase of stem branching and in the phase of flowering in order to make it possible to trace the daily dynamics of the carbohydrates. Plants, 80-120 in each sample with two repetitions (joining plants from two plots in one repetition), were cut at ground level, the leaf blades were separated from the remaining aerial organs, and (for the phase of stem branching) the uppermost undeveloped leaf was discarded; the green and then the air-dry weight of these were measured. The material was fixed with steam for 15 min, dried to a constant weight at 40° and carefully ground. Extraction was carried out by boiling with 80% alcohol, the alcohol was distilled off with a vacuum at 35-37°, and the viscous remains after washing with petroleum ether were transferred to water, purified of

TABLE 1. Data of Phenological Observation of Wheat in 1955 Experiments

time of observation	Moscow	Apatity	
		natural day	12-hour day
sowing	May 22	June 3	June 3
sprouts	May 29-30	June 11	June 11
appearance of fourth leaf	June 11	June 24	June 30
taking samples in period of stem branching	June 17-18	July 9-10	July 9-10
flowering (and taking samples)	July 15-16	Aug. 18-19	flowering did not begin

proteins, concentrated under phenol to dryness and transferred to 80% alcohol.

Investigations of the carbohydrate composition of wheat leaves carried out at the beginning according to the scheme of Kizel' showed that there are no essential variety differences for the wheat used in the experiments in relation to all fractions: In all cases the alcohol-soluble carbohydrates represented, in the main part, fractions of the easily hydrolyzed nonreducible sugars, the total concentration of which is 3-4% on the average, and in some cases approaches 12-14% of the dry weight of the leaves.

The concentration of carbohydrates of this fraction is changed sharply as the day passes (from a maximum about 5 P.M. to a minimum at 4-5 A.M.) and also in relation to the phase of development of the plants (see also Kokin [1]; Vasil'ev [2]). In the leaves of young plants cultivated in Zapolyar'e on natural day, the fraction of "disaccharides" is especially abundant.

With the use of the paper chromatography method, we decided to determine if all changes of the indicated fraction are related in proportion to sucrose and which other components enter into the composition of the fraction of easily hydrolyzed alcohol soluble oligosaccharides of wheat leaves.

Many researchers, measuring the indicated fraction by the Bertran method, speak of it as sucrose [3-5]. Even at the end of the last century and the beginning of the present one, it was established that not only the grain, but also the leaves of a number of grasses (wheat, barley, oats and others) contain easily hydrolyzed water soluble carbohydrates at the end of the vegetation period, giving fructose as a predominant component on acid hydrolysis, and therefore possessing fructosans. A thorough survey of these early works was made by Kizel' and Kretovich [6], who observed the appearance of substances the acid hydrolyzate of which possessed a counter-clockwise rotation in all aerial organs of rye from the beginning of flowering. Work in recent years [7] also supports the observation that fructosans, and in particular, glucofructosans, are observed only at the end of vegetation in the phase of flowering and after in the leaves of summer wheat, and only for winter wheat are they present over the entire summer period beginning with early spring [8].

Actually, as the investigations of a number of authors [9] showed, a number of other alcohol soluble oligosaccharides besides sucrose are accumulated in the leaves of winter wheat in the process of hardening.

With respect to summer wheat, until recently it was not clear how the composition of the fraction of soluble carbohydrates is changed in the leaves with reference to age, phase of development, time of day and external conditions, in spite of the growing interest in this question [10-13]. Our investigations were devoted to an explanation of several questions of the problem indicated.

The method of paper chromatography is a very convenient means of observing qualitative differences in the composition of extracts from plant tissues, but the analysis of eluates of the chromatograms allows us also to give a quantitative appraisal of the different components of these extracts. We carried out our investigations using the method of descending paper chromatography on Leningrad fast paper and a mixture of butanol–glacial acetic acid–water (4:1:5), with development by reagents to a ketose (naphtharesorcin-trichloroacetic acid, urea) and also an aldose analine phthalate [14, 15].

Quantitative measurement in the eluates of the chromatograms was done with an anthrone reagent [16]. In those cases where the concentration of individual components of the oligosaccharide fraction was low, the results of the individual measurement were checked by means of two to three neighbors, and sometimes also of all chromatographic zones of the oligosaccharides (with the exception of sucrose).

Stem Branching Phase. A significant difference for the plants in the phase of stem branching between cultivation in the conditions of Zapolyar'e and Moscow was already observed in the first experiments based on chromatography (see chromatogram). This difference consists in the presence in the leaves of the northern wheat variants, especially on the natural day, of a relatively large quantity of a number of alcohol soluble oligosaccharides, distributed on a descending chromatogram above sucrose, than is, with an $R_{sacch.}$ less than unity. The less mobile of them apparently possess a more complex molecule than sucrose [13, 17].

The coloring of the spots of the oligosaccharides, denoted by us in the order of their position behind sucrose (zone 1) as zones 2, 3, 4, 5, 6, and 7, showed that they are related to the glucofructosan group [13]. The character of the connections and relationship of monose precipitates have not yet been investigated by us. We separated, in addition to sucrose, seven zones of oligosaccharides; however, there are indications that several spots on the chromatograms can be created not of one,

Fig. 1. Descending paper chromatogram of oligosaccharides of wheat leaves at the beginning of the stem-branching phase. Solvent: n-butanol–acetic acid–water (4:1:5), 96 hours; A) Moscow; B) Apatity, natural day; 1) sucrose; 2-7) oligosaccharides (developed with naphthoresorcin).

TABLE 2. Alcohol Soluble Carbohydrates of the Leaves of Khibiny 314 and Lyutestsens 62 Wheat During the Stem Branching Phase at Different Hours of the Day (in mg per g of dry leaves)

component	Moscow		Khibiny 314				Lyutestsens 62				
	Moscow		Apatity, natural day		Apatity, 12-hour day		Moscow, 5. P. M.	Apatity, natural day		Apatity. 12-hour day	
	June 17, 5 P. M.	June 18, 5 A. M.	July 9, 5 P. M.	July 10, 5 A. M.	July 9, 5 P. M.	July 10, 5 A. M.		5 P. M.	5 A. M.	5 P. M.	5 A. M.
fructose	3.1	1.2	3.0	3.4	2.1	2.7	2.0	3.0	3.6	1.8	2.0
sucrose	66.4	11.8	74.0	40.0	58.2	13.7	57.0	72.0	35.0	52.9	20.0
2	2.2	1.1	10.0	8.2	4.0	1.5	0.9	9.8	7.2	3.8	2.0
2a	absent		1.3	2.2	absent		absent	3.2	1.6	absent	
3	1.0	1.1	2.5	3.3	1.6	0.9	1.2	5.6	3.8	2.0	2.0
4	trace		7.7	9.9	3.7	2.0	trace	10.4	7.5	2.0	2.3
5	trace	trace	3.7	5.9	2.1		"	9.8	7.0	1.1	1.5
6	"	"	2.3	4.2			"	5.2	3.7	1.1	0.8
7	"	"	3.6	7.6			"	21.0	11.3		1.5
total oligo-saccharides (less sucrose)	3.2	2.2	31.1	41.3	11.4	4.4	2.1	65.0	42.1	10.0	10.1

but, to a slight degree, of two to three components [18, 19].

In the Moscow experiment (Table 2) the indicated oligosaccharides in the phase of stem branching are nearly absent, with the exception of two of the most mobile components (zones 2 and 3), which are distributed on the chromatogram next to sucrose. However, these oligosaccharides are also present in very small quantities, on the order of 1 mg per 1 g of dry weight of the leaves.*

On the natural Polar day, wheat leaves, as we already stated, accumulate a fairly significant quantity of alcohol soluble oligosaccharides, constituting chromatographic zones 2-7. As many as half of the total number of alcohol soluble sugars of wheat leaves of the long day variant belong to the portion of this group of compounds, and the total concentration of the mentioned oligosaccharides constitutes 30-40 and even up to 65 mg per 1 g of the dry weight of the leaves. The most abundant components both for Lyutestsens 62 and Khibiny 314 are 2, 4, and 7, at all hours of the day, and this corresponds to what was found earlier [13] for the period immediately before flowering. With respect to the character of the diurnal dynamics of the oligosaccharides, with the exception of sucrose, one cannot say anything specific at the present time because in some cases these substances were observed less in the early morning hours (Lyutestsens 62 in the stem branching phase, Table 2) than during midday hours, but in other cases the opposite picture was observed (Table 2). The concentration of component 7 was significantly higher in the leaves of Lyutestsens 62 than in Khibiny 314.

The restriction of the light period of the day to 12 hours caused a significant decrease in the concentration of all alcohol soluble oligasaccharides in the leaves of wheat during the phase of stem branching at all hours of the day; this decrease concerned the chromatographically least mobile components (zones 5, 6, and 7). The qualitative composition of the oligosaccharide fraction in the leaves of both varieties of wheat in the short day variant was practically the same as on the natural Polar day. The difference was in the fact that on the 12-hour day in the stem branching phase the component designated zone 2a by us (Table 2) was completely absent at all hours of the day both for Lyutestsens 62 and for Khibiny 314. We must note that component 2a was also absent in the stem branching phase in the wheat leaves of the Moscow experiment.

The concentration of sucrose under the conditions of the full Polar day at all hours was significantly higher than in the Moscow experiment. Thus, at 5 P.M. (the time when the sucrose in the wheat leaves is at a maximum), in the first case it was 72-74 mg per g of dry leaves, against 57-66 mg in Moscow. The concentration of sucrose in the leaves always decreased sharply toward morning, reaching a minimum at 5 A.M.; thus, a sharp diurnal rhythm was observed. This decrease over the night hours in absolute figures is 34-37 mg on the natural day in Zapolyar'e and 45-55 mg in Moscow. Thus, a greater quantity of sucrose is retained in the leaves of the first of the indicated variants towards the morning.

* The word "trace" in the tables opposite other zones shows that in the investigated plant material the concentration of oligosaccharides measured by us, if they generally are present, do not exceed 0.05% of the dry weight of the leaves; that is, they are present in the range of the error of the experimental method.

TABLE 3. Alcohol-Soluble Carbohydrates of Leaves of Khibiny 314 Wheat in the Flowering Phase at Different Hours of the Day (in mg per g of dry leaves)

component	Moscow		Apatity, natural day	
	5 P.M. 15.VII	5 A.M. 16.VII	5 P.M. 18.VIII	5 A.M. 19.VIII
fructose	2.5	0.8	6.5	2.6
sucrose	42.0	12.7	37.0	9.0
2	0.9	trace	1.1	1.2
2a	0.6	0.5	1.3	1.9 (2a–3)
	(trace)	(trace)		
3	0.8	1.0	1.3	
4	0.7	0.8	2.3	2.5
5	0.8	0.8	1.8	7.9 (5–7)
6	0.5	1.6 (6–7)	1.1	
	(trace)			
7	1.9		6.5	
total oligosaccharides (less sucrose)	6.2	4.7	15.4	13.5

As a result of the restriction of the light period of the day to 12 hours, the concentration of sucrose both in the evening and morning hours appeared to be approximately the same for the North as in Moscow.

Of the monoses we determined the presence of fructose and glucose, which are contained in wheat leaves in approximately equal quantities, to be on the order of 1.5-3.5 mg per g of dry leaves (only the data for fructose are presented in the tables). The indicated monosaccharides do not experience specific diurnal dynamics, which agrees with the notes in the literature [1,19] and according to our data, a clear relationship is not observed between their concentration in the leaf tissues of wheat and the conditions in which the plants were grown in the Moscow and North variants.

Flowering Phase. Wheat plants did not flower under the conditions of a 12-hour day, and so only natural day variants were investigated in the flowering phase. Because both of the wheat varieties used in the experiment, Khibiny 314 and Lyutestsens 62, appeared to be very similar in the carbohydrate composition of the leaves at all hours of the day, we will present data only for variety Khibiny 314 (Table 3).

The results of the quantititave measurements show that the total concentration of alcohol-soluble oligosaccharides on the natural day in Zapolyar'e both in the evening and morning hours is significantly lower in the flowering phase than in the stem branching phase. Other authors [20] also show the exhaustion of the leaves of grass plants in the flowering period. At the same time we must consider that there is an actual night duration of about five hours in the Murmansk region in the period of flowering (samples taken on August 18-19), and this can partly determine the low morning level (about 9 mg per g of dry weight) of the concentration of sucrose. The concentration of other oligosaccharides at all hours of the day also decreased in the flowering phase.

In the Moscow experiment, the leaves of wheat plants in the flowering phase acquired the ability to accumulate small but sufficiently well observed quantities of components 4-7, on the order of 1 mg per g of dry weight. At the same time, they are much less numerous than in the same phase of flowering for plants in the North, Besides this, traces of component 2a (about 0.5 mg) appear in wheat leaves of the Moscow variant in this period. The concentration of sucrose on the leaves of these plants is decreased in relation to the stem branching phase only during the daytime hours (approximately by 35-40%), while the early morning level remained as before.

DISCUSSION

The data presented above make it possible to some degree to judge the extent of the qualitative and quantitative changes of sugars, and primarily oligosaccharides, in the leaves of summer wheat under the effect of different external conditions, and also in connection with the development of the plants. In the investigations published up to the present time, also using the paper chromatography method, the predominance of sucrose is shown in summer wheat leaves in all vegetation periods, with the exception of the first days of germination when a significant part of the soluble sugars are monosaccharides, and the appearance of small quantities of substances from the oligosaccharide group is noted only at the end of the life cycle of the plants.

Glucofructosans were not observed in any noticeable quantities (in the beginning phases of the development of summer wheat) either by chemical or by chromatographic methods in any of the investigations [10,21]. We were able to explain that in the conditions prevalent in the Moscow experiment, alcohol soluble oligo-

TABLE 4. Analysis of the Soil from Different Plots of the Experimental Field, Kolya Section, USSR Academy of Sciences, in the Apatity Region

sample No.	humus	ash	calcium	pH	K_2O, mg per 100 g of soil	P_2O_5, mg per 100 g of soil: soluble in citric acid	water soluble	soluble in acetic acid
	% of absolutely dry weight							
1	4.52	89.0	0.72	5.97	52.3	17.6	none	1.09'
2	6.72	84.4	0.48	6.08	19.7	27.0	none	2.03

saccharides with a molecule containing more than two to three monose residues are nearly absent in the stem branching phase of summer wheatleaves, and from the simple sugars we find smaller quantities of fructose and glucose (approximately 1-3 mg of each per g of dry leaves), significant quantities of sucrose (up to 5-6% of the dry weight of the leaves) and also two low molecular glucofructosans (1-2 mg of each) of the number appearing on the chromatogram next to sucrose components 2 and 3, as named here.

However, when wheat was grown in the conditions of the field experiment in Zapolyar'e, we observed in the leaves in the same phase of stem branching, in addition to sucrose (its concentration was even higher than in the Moscow experiment), very significant quantities differing in their chromatographic features, and judging by the complexity of other oligosaccharides and the character of the coloring by specific reagents, there are also glucofructosans.

In connection with the fact that the experiments were carried out under field conditions, we were not able to calculate the entire complex of the climatic conditions to explain precisely which factor of the complex determines the observed differences in the carbohydrate composition of the wheat leaves. It is entirely probable that this is the result of the action of not one, but a number of factors of the external environment, the intensity of which is very different in those two geographical points where our experiments were carried out. Here we refer to the different conditions of illumination (length of day, intensity of lighting), moisture and temperature of the soil and air, and also the important differences in the composition and structure of the soil (Table 3).

In the 1955 experiments, we investigated only the effect of the length of day by creating a dark period in the Zapolyar'e conditions. We must note, however, that the 12-hour day, which was used for wheat plants, is completely inadequate for them as long-day plants, and this also caused a retardation in flowering as compared with the variant with day round lighting. At the same time, the creation of a 12-hour dark period led to a significant (approximately 50%) decrease in the concentration of the total of alcohol soluble oligosaccharides.

However, qualitative changes were not observed with significant quantitative shifts of the carbohydrate solution in the leaves of the young wheat plants in the North under the effect of a shortened day (or the addition of a dark period), with one exception: In this case component 2a was absent during all hours of the day. The fact that one component was missing in the leaves in the stem branching phase and in the Moscow experiment suggested a change in the means of converting oligosaccharides and, in particular, glucofructosans, under the effect of the dark period. The idea developed of the different methods of synthesis, and also of the use in plants of oligosaccharides, differing in complexity (or configuration of the molecule), even if their molecules consist of residues of the same monose (for example, in the case of glucofructosans). Such a proposition was suggested by Bacon [22], Schlubach [23], and also in Bourdu's work [24]. In this connection, we must note that it is possible that several enzymes, invertase primarily [19,26], are capable of synthesizing on a sucrose base only oligosaccharides with a short bond (three to four monose groups), while other enzymes synthesize more complex oligosaccharides with a bond of six to seven ring and more, judging from the data presented in the review work of Edelman [25].

Light intensity can show an effect on metabolism in the plant [27], reaching a maximum in clear weather at 55° north latitude of 80-90 thousand lux and not exceeding 45-50 thousand lux in Zapolyar'e. Besides this, we must consider that the number of cloudy days is greater and the number of clear days fewer in Zapolyar'e than in the middle band of the European part of the USSR.

Temperature also affects the activity of the plant organism greatly. It is true that the minimum temperature at the time that the samples were taken in the phase of stem formation in 1955 was 14-15° in Moscow and 12-13° in Apatity, that is, the difference was not great, about 2-3°. However, the average minimum temperature both in the period of sprouting to stem formation and especially in the period of stem formation to flowering was significantly lower in Zapolyar'e than in Moscow.

In addition to what has been said above, we must remember that in our 1955 experiments, similar conditions were not created in the Moscow and Northern plant groups either in regard to mineral nutrition, an area in which a good deal of work [21,28] has been devoted to explaining the role of the carbohydrates in the metabolism [29].

Thus, from all that has been said above, it is obvious that the essential differences that we observed in the composition of the fraction of alcohol soluble oligosaccharides in wheat leaves in the early phases of plant development can exist as a result of the complex interaction of the various factors of the external environment. An explanation of the role of several of these factors is a goal of our future investigations.

SUMMARY

Under certain conditions of growth, summer wheat plants are capable at an age of two or three weeks to store up in their leaves significant amounts of oligosaccharides, other than sucrose, which belong to the glucofructosan group. This was especially noticeable in field wheat grown at the far North (Kola Peninsula). At the beginning of the stem branching stage not less than 7

components of the indicated type of oligosaccharides were detected in the leaves, and in some cases their total amount was the same as the sucrose content, which is 4-6% of the dry weight.

Shortening of the day length in the northern experiments to 12 hours led to a considerable reduction of the concentration of all types of oligosaccharides in the leaves of young wheat plants but almost did not change the qualitative composition of this group of substances.

During the corresponding developmental phases of wheat grown in moderate regions (Moscow), besides sucrose only two other low molecular glucofructosans have been detected in the leaves, the amounts of these substances being lower than 0.1-0.2% of the dry weight. However, during the flowering stage these plants also become capable of accumulating apparently all glucofructosans found in the early developmental periods in wheat grown in the north.

The concentration of all investigated glucofructosans varies during the day and night, but a rhythmic diurnal variation has been found to be characteristic only of sucrose.

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† See English translation.

THE RELATION BETWEEN CHANGES CAUSED BY DROUGHT AND INFECTION OF PLANTS BY PUCCINIA TRITICINA

T. G. Kydrev and L. A. Tyankova

Institute of Horticulture, Bulgarian Academy of Sciences, Sofia
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Many investigators have attempted to find a connection between the action of drought on plants and their infection by different fungus diseases [1-3]. Thus, Stakman [3] observed that Puccinia triticina Erikss develops better on drought resistant plants when they are growing on dry soils, and on mesophytes on moist soils. Clayton [2], while investigating fusarium blight of tomatoes, came to the conclusion that this disease heavily infects those plants that were grown under conditions of normal moisture during the beginning period of development and were subjected to drought after this. Plants that were grown under conditions of relatively low soil moisture from the very beginning of their development appeared to be more resistant to fusarium. Plants growing on moist soil appeared to be immune. Saburova (cited by Kokin [4]) shows that wheat plants are most heavily infected by rust on high soil moisture and most weakly on low soil moisture.

One of the authors [1] studied in more detail the effect of atmospheric drought on the infection of wheat with P. triticina. Plants growing under normal conditions of nutrition and soil and atmospheric moisture were infected with rust and then subjected to the action of artificial drying. As a result, the period of development was shortened, the formation of uredospores accelerated and the number of pustules of the fungus significantly increased. For several varieties the differences that were observed in the beginning of development disappeared with time, but they were retained in other varieties. On the basis of these data the author concluded that drought shows a favorable effect on the development of the rust infecting the plant. Evidently, in this case the favorable effect of the drought is not immediate, but appears through the action of the plant-host. It appears that a change aiding the development of the uredospores takes places under the effect of atmospheric drought in the plant organism.

In this connection, we had as our objective the explanation of the degree to which changes caused by drought conform to those characteristics of the plant that favor their infection with the rust. Along with this, we will also examine the leaf changes based on the level of the leaves in connection with their susceptibility to infection.

We used wheat plants of Bulgarian variety 301 after their earing out to carry out the present investigation. We determined the type and degree of infection of the plants subjected to drought and of the control plants, the number and dimensions of the stomata, the intensity of transpiration, the permeability of the plasma, the concentration of protein and nonprotein nitrogen, and that of growth and tanning substances.

Plants were inoculated with a mixture of different races of rust, Puccinia triticina Erikss, for which uredospores were mixed in a 0.3% solution of gelatin. Plants were sprayed with this solution and then were transferred to a moist innoculation chamber. The walls of the chamber were sprayed with water during the day to keep a high relative humidity in the chambers. The plants were kept in such conditions for 48 hours, after which they were removed from the chamber and grown at a temperature of about 20° with normal soil temperature. Observations were made daily. The results were judged on the fourteenth day after innoculation.

The number of stomata was established microscopically. The epidermis from the lower surface of the middle part of the leaf was stripped off and a section of this was observed under a microscope. The dimensions of the stomata were determined with a micrometer. Data on the number of stomata by leaf level and their dimensions are average figures obtained from measurements in four visual fields.

The intensity of transpiration of the leaves was determined by the cobalt method at 25° during the middle hours of the day simultaneously for all leaves. Separate measurements were made in four repetitions.

The permeability of the leaves was determined by the Sukhorukov method [5] and the concentration of nitrogen by the Kjeldahl method.

Auxins were obtained by the extraction method and their identification made by testing on oatmeal cylinders.

Tanning substances were identified by the method described in the work of Belozerskii and Proskuryakov [6].

TABLE 1. The Number and Dimensions of the Stomata, Area of Leaf Surface and Intensity of Transpiration by Levels

level of leaves from top down	number of stomata in one visual field	dimensions of the stomata, micron		leaf surface, cm	intensity of transpiration, minutes
		length	width		
first	18	58.68	38.64	16.5	2
second	15.5	62.90	45.88	23.3	3
third	14	71.09	50.67	19.3	4

TABLE 2. Organic Substances Washed Out of Leaves of Different Levels of Wheat Plants

level of leaves from top down	quantity of organic substances, in ml of 0.1 N $KMnO_4$	
	control	drought-subjected
first	0.1	1.5
second	0.4	3.2
third	1.8	3.5

Artificial drought was obtained by the method described in [1].

The period of development for the plants subjected to the effect of drought and then innoculated with the rust was shortened at this time, and the development of uredospores on them was accelerated; opening of the pustules took place for the experimental plants after six days, but for control plants after eight days. The type of infection was the same. On the tenth day a sharp difference was observed in the degree of infection, which for the dry plants was appraised as mark 3, and for the control plants ranged between 1 and 2. On the fourteenth day these differences were practically obliterated. Besides this, visible differences were noted in the degree of disease of the plant leaves by level. On the uppermost leaf, the growth of which had not completely stopped, there were hardly any pustules to be found. On the following leaf the pustules appeared in large numbers. It was characteristic that on these two leaves more pustules were formed on the upper part of the leaves than on their base. For the following levels of leaves, differences in the degree of infection and also in the distribution of pustules on the various parts of the leaf were not observed. Under the effect of drought, the tip parts of the leaves dried out. At the same time a greater number of pustules was observed in living tissues in immediate proximity to the upper part. Differences in the distribution of pustules on leaves of different levels of the wheat plants were clearly expressed for the plants in dry conditions.

The observed features of the appearance of pustules on experimental and control plants shows that drought actually aids infection by the rust. It is natural that the reasons for this effect must found in those changes that take place in the plant under the effect of drought. At the same time characteristic differences in the degree of infection of the different levels makes it possible to find factors in their physiological features affecting the infection. Below is an attempt to explain several of these features and changes.

Anatomical-morphological features of plants have an important effect on the plant's adaptation to the unfavorable effect of drought. Several authors stress the fact that these features have importance also for the resistance of plants to rust. According to Cobb's theory [7] on "mechanical immunization", plants with a thick cuticle, waxy deposits, small stomata and very pubescent upright leaves are resistant to rust. Allen [8] considers that stomata play an active role in the resistance of plants to rust. Hart [9] explained a relationship between the collenchyma and sclerenchyma tissue and the formation of rust pustules. However, other authors [10,11] deny the existence of a connection between anatomical-morphological features of plants and their rust resistance. We actually observed a number of such features for leaves of the lower levels, and this will be discussed below.

We know that the external conditions show a very strong effect on the formation of any anatomical-morphological structure [12,13]. In this connection, the location of the leaves by level on the plant greatly

affects their structure and physiological functions (Table 1).

The data in Table 1 show that the differences between the number and dimensions of the stomata, and also the surface of the leaves and their transpiration, are obvious. These differences characterize the dissimilar drought resistance: higher for the upper and lower for the lower levels of leaves. With an inadequate water supply, the upper leaves are able to take away part of the water supplies from the lower leaves and thus cause their more significant water deficit [13]. In this manner one can expect that certain physiological changes take place in the lower leaves as a result of the water-using action of the upper leaves, and those features become characteristics by which plants differ in withstanding drought. A number of other features are actually observed for the leaves of the lower levels and will be discussed below. The more mesophytic structure of the lower leaves, along with their physiological specifications, obviously also favors the more intensive development and formation of uredospores on them.

One of the important characteristics of protoplasm is its permeability. Sukhorukov [5] and Kokin [4] discovered that a specific inverse relationship exists between the permeability of protoplasm and the resistance of plants to infection. They also note that after infection, the permeability of the plant cells increases sharply.

Concerning the question of the effect of drought on the permeability of the protoplasm, we know that a specific relationship exists between them. According to Maksimov [13], one of the first appearances of the infection of protoplasm under the effect of drought is an increase in its permeability. A short period of drought leads to an insignificant increase in permeability, which disappears easily and rapidly after the reestablishment of normal conditions. Prolonged germination causes an opposite or nearly opposite increase in permeability, which in the end leads to the death of the cell.

The results that we obtained on measuring permeability are given in Table 2. Table 2 shows that the permeability of the leaf plasma by levels increases from top to bottom.

The effect of drying is sharply expressed in the increase in the permeability of leaf plasma. The permeability of the plasma of the first leaf of plants subjected to drought (experimentsl plants) is fifteen times that of the permeability of the first leaf of control plants. The figure for the permeability of the first leaf of experimental plants is approximately the same as that of the third leaf of control plants; the second leaf is 32 times and the third 35 times that of the permeability of the first leaf of control plants.

There are two periods characterized by a sharp increase in the permeability of the plasma of the cells in the development of wheat in the conditions of Bulgaria during the spring and summer. These are the periods of young age: The first ends in the middle of spring and the second in the period of flowering. It is characteristic that very low resistance to drought is observed for plants at this time [14]. According to investigations of a number of authors [15,16], wheat plants most easily give in to infection by rust at a young age and at the time of flowering.

The coincidence of the periods when wheat displays an increase in drought resistance with the periods of increased resistance to rust shows the presence of a connection between these two factors.

There are indications of the great importance of auxins in the resistance of plants to fungus diseases. Gentil and Klein [17] note that indoleacetic acid when applied to the plant retards more than stimulates the development of the fungus. Défago [18] asserts that a known connection between the resistance to disease and the quantity of indolacetic acid exists.

The question of the role of auxins in the increase and decrease of drought resistance must be considered insufficiently clear and weakly developed.

The results that we obtained of the measurement of auxins are presented in Table 3.

In our experiment the quantity of auxins in the leaves decreases from the top down. As a result of the effect of drying, the quantity of auxins in the plants is decreased.

On the basis of the data that were obtained, one can assume that the effect of drought, leading to a decrease in the quantity of auxins, indirectly favors the infection of plants with fungus diseases.

Several authors connect the resistance of plants to rust with characteristics of their nitrogenous substances. According to data in the literature [19], a direct relationship between the quantity of proteins and the resistance of wheat to rust exists. Gassner and Franke [20] consider that an inverse relationship exists between resistance to rust and the quantity of the albumin fraction of proteins.

TABLE 3. Concentration of Auxins in Wheat Leaves by Level and in Entire Control and Experimental Plants

level of leaves from top down and entire plants	lengthening of the oatmeal cylinders	
	in mm	as % of first leaf or control plant
first	2.11	100
second	1.49	70.61
third	1.40	66.39
entire plant:		
control	1.85	100
experimental	1.32	71.35

TABLE 4. Concentration of Total, Protein and Nonprotein Nitrogen in Wheat Leaves by Level and in Entire Control and Experimental Plants (in mg per g of dry substance)

Level of leaves from top down and entire plants	Nitrogen total	nonprotein	protein	Relationship of nonprotein and protein nitrogen as of % of total
first	20.70	4.37	16.32	21.14: 78.86
second	20.38	7.50	15.08	26.72: 73.23
third	15.16	4.76	10.40	31.39: 68.61
entire plants:				
control	28.02	6.162	21.86	22.12: 77.88
experimental	23.56	12.48	11.07	56.60: 43.40

TABLE 5. Concentration of Tanning Substances in the Separate Levels of Leaves and in Entire Control and Experimental Wheat Plants (in mg per g of dry substance)

level of leaves from top down and entire plant	tanning substance in mg of tannin	as % of first leaf or control plant
first	21.580	100
second	13.487	62.5
third	9.441	44.0
entire plant:		
control	18.660	100
experimental	1.492	8.0

A number of existing questions connected with the metabolism of nitrogenous substances in control plants and plants exposed to drought were developed in detail by Mothes [21] and Sisakyan [22]. It was established that drought leads to a disintegration of the protein substances of the plants.

The results of our measurements of the total, nonprotein, and protein nitrogen are presented in Table 4.

As Table 4 shows, the concentration of total nitrogen in the leaves is gradually decreased from the top down. Protein nitrogen also decreases in the same direction. The relation of nonprotein and protein nitrogen as percentages of the total is very characteristic. In the highest (first) leaf the quantity of nonprotein nitrogen is only a third of the protein nitrogen. In the following levels there appears to be a tendency toward an equalizing of the relationship of the two forms of nitrogen, with the concentration of nonprotein nitrogen increasing and the protein nitrogen falling. In the third leaf the quantity of nonprotein nitrogen is approximately half that of the protein nitrogen.

In our experiment the effect of dryness was a considerable decrease in the quantity of protein and increase in nonprotein nitrogen. With this, the quantity of protein nitrogen remained less than that of the nonprotein. On the basis of the data that were obtained one can conclude that drought favors the development and formation of uredospores also by means of the disintegration of the proteins of the plant host.

In connection with the resistance of plants to rust, tanning substances are of especially great importance. The investigations of Cook [23] and Kargopolova [24] showed that a direct relationship exists between the quantity of tanning substances and the resistance of plants to rust.

We were not able to find in the literature original data on the relationship between the resistance of plants and the concentration in them of tanning substances

Our experiments showed that as a result of drought the quantity of tanning substances in plants falls sharply (Table 5). A decrease in the quantity of tanning substances in plant tissues favors the infection of wheat with rust.

SUMMARY

Investigations of many authors show that an increased permeability of the cell protoplasm, a decreased concentration of auxins, an increased quantity of nonprotein nitrogen and a decreased quantity of tanning substances favor the infection of wheat plants with rust [4, 5, 17-19, 23, 24].

As a result of the effect of artificial drought, the permeability of the cell protoplasm and the quantity of nonprotein nitrogen was increased, but the quantity of auxins and tanning substances was decreased. Thus, changes caused by drought coincide with those features of plants that favor their infection with rust. Consequently, the effect established by Kydrev of drought (artificial drought) on the shortening of the period of development and formation of uredospores, and also the more intensive appearance of pustules on the leaves of the lower levels, which we established, can be explained by the indicated disturbances in metabolism which favor the development of the rust. An increase in the pustules on the lower levels, along with the disturbances in metabolism mentioned above, are also probably aided by a more mesophytic structure of these leaves.

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APPLICATION OF A METHOD FOR CULTIVATION OF ISOLATED TERMINAL BUDS TO STUDY THE PROCESS OF GROWTH AND ORGANOGENESIS OF PLANTS

R. G. Butenko

K. A. Timiryazev Institute of Plant Physiology, Academy of Sciences of the USSR, Moscow
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The growth and organogenesis of plants is one of the most complex and integral processes of the plant organism. The nature of this process, which is specified by the inherited characteristics of the plant to a significant degree, depends in its concrete existence on a number of external ecological factors, among which temperature, length of day, and the intensity and spectral composition of light play an important role. Changes in the path of the plant's organogenesis caused by external or by internal effects are determined in the end by the characteristics of metabolism in the meristem of the stem buds.

It is difficult in the complex system of the entire plant organism to separate the direct effect of ecological factors on the meristem of the terminal buds from the effect to which the meristem is subjected as a result of changes in the physiological activity of other organs, which are reacting in relation to a particular factor. It is still more difficult to determine the character of concrete products of metabolism, through which the effect of the prescribed organ on the meristem exists. One of the methods encompassing a resolution of these questions is the use of the method of the cultivation of isolated terminal buds of plants on artificial nutrient mediums.

White [1], in his well known summary on tissue culture, writes that stem meristems of plants are unsuitable as a culture object because they form roots and leaves quickly and become minature copies of the entire plant. This feature of stem meristems obviously is also a reason for the fact that there are relatively few studies on the cultivation of terminal meristems in the vast literature on the culture of isolated tissues and organs of plants.

In 1922 Robbins [2], one of the developers of the method of culturing plant tissues, attempted to cultivate stem tips of beans, corn, and cotton with lengths from 1.45 to 3.75 cm on an artificial nutrient medium. After 11-20 days, the tip cultures increased in dimensions, forming several small chlorotic leaves and developing roots. White [3] attempted to grow in vitro in a suspended drop very small tips (0.1 mm and smaller) of the terminal meristem of chickweed (Stelaria media L.). The tissue lived for a period of three weeks, and cell divisions were observed in it, but it did not form an entire little plant. La Rue [4] cultured in vitro tips of stem tissues consisting of the base of the cotyledon and terminal bud. These buds formed shoots and roots. Smith [5] studied the effect of trace elements and vitamins on the growth of isolated tips of sunflower stems.

Loo [6], while growing tips of dodder stems in vitro, observed the formation by an explant of bean buds and flowers without the formation of leaves and roots. In other experiments by Loo [7], isolated stem tips of asparagus were grown on an artificial nutrient medium for a period of 20 days by culturing isolated roots. There is a great deal of interest in the experiments of Kawata [8], who first obtained the formation of an ear and brush in a culture of isolated stem tips of wheat and rye. The authors of other studies that were made with very small pieces of stem meristems had as their objective the explanation of whether the meristem of the growth cone of the stem, independent of geographical locality, is capable of the process of organogenesis without other parts of the plants, or if its growth and development is closely connected with stimulants proceeding from other organs.

To resolve this problem, Ball [9,10] cultured pieces of the apex of lupine and nasturtium with dimensions of 55 x 140 microns on an artificial nutrient medium and compared their behavior with respect to the organogenesis of the strictly meristematic region of the growth cone and of the differentiated tissues lying below. It was shown that under certain conditions of the cultures of only the strictly meristem tissue capable of organogenesis, the differentiated tissues gave callus-like growth. Ball considers that stem meristem potentially is capable of organogenesis also in an isolated condition, but this capability is closely connected with the activity of the remaining organs. Morel [11] grew very small pieces of the growth cone of dahlia and potato and observed that in most cases such tissue is capa-

ble only of nondifferentiated, tumorlike growth, and only the addition of gibberellic acid, vitamins, and kinetin to the nutrient medium caused slight differentiation.

In our experiments we studied the features of the transfer of isolated terminal buds of a number of plants from vegetative to generative condition under the effects both of physical conditions of growth and of nutrients obtained by them from the nutrient mediums. In this connection, terminal buds of adult, vegetating plants were used in the experiments, which demanded special methods of sterilization of tissues and selection of conditions favorable for their cultivation.

MATERIALS AND METHODS

Experiments on culturing isolated terminal buds were carried out at the Artificial Climate Station of the USSR Academy of Sciences Institute of Plant Physiology during 1958-1960. Terminal buds of adult, vegetative plants of red perilla (Perilla nankinensis), rudbeckia (Rudbeckia bicolor), Kharbin soy (Soja hispida), blue annual lupone (Lupinus angustifolius) and two species of tobacco, Nicotiana tabacum var. Maryland Mamont and N. silvestris, were used. All of these plants are photoperiodically sensitive and were grown on an unfavorable day length in order to keep them in a vegetative condition. Isolated terminal buds were sterilized by various sterilizing solutions prior to being planted on the artificial nutrient medium. Most authors whose works have been mentioned in the introduction avoided the sterilization of the meristem by growing the entire plant in sterile conditions from previously sterilized seeds. Several of them [8,9,10] cultured tissue of strictly meristem without leaf rudiments, but aseptic conditions are usually maintained in the meristem, and if one is careful while isolating, special sterilization is not needed.

In our experiments, where the terminal bud of the mature plant, including leaf rudiments with the meristem, was isolated, the moment of sterilization of the plant tissue itself was difficult, but completely necessary. Calcium hypochlorite (7.1%), chloramine (10% solution based on active chlorine), hydrogen peroxide (10-12%), antibiotics (1-4 mg/liter), diacid (ethanolmercurochloride + boric acid with cetyl pyridine bromide as a wetting agent) and a solution of mercuric chloride (0.1%) were tried as sterilizers. Mercuric chloride gave the best results for all plants used in the experiments in the sense of effectiveness of sterilization with high preservation of the plant tissue. The time of sterilization with mercuric chloride varied significantly for the various plants. The optimal sterilization period of terminal buds was three minutes for red perilla, three minutes for soy, four minutes for lupine, 12 minutes for Sylvester tobacco, 15 minutes for Mamont tobacco, and eight to nine minutes for rudbeckia. However, none of the tested sterilizing solutions or methods of sterilization gave satisfactory results for tips of Sylvester tobacco and rudbeckia. The reason for the poor sterilization of these particular plants was internal microflora not sensitive to external sterilization and adapted to tissue of root origin. Isolation of the stem bud of a rosette form for rudbeckia and Sylvester tobacco on an unfavorable day length is inevitably connected with the presence in the planted bud of tissues of the root collar and roots, which are also reasons for further infection. The addition to the medium of a number of antibiotics (streptomycin, biomycin, terramycin) in our case did not improve the problem, causing only simultaneous oppression of the growth of the plant tissue. The corresponding preparation of plants prior to the isolation of the buds appeared to be more favorable for rudbeckia. Plants having a rosette form as a result of the short day were shifted on the seventh day into long day conditions and after some elongation of the stem were again grown on the short day until a stable rosette was secured.

Such a method aided in raising the point of growth of the rosette above the ground and establishing it wholly on the stem parts of the plants. Tissues of the terminal buds of the rosettes raised above the ground were infected to a significant lower degree, and in this case sterilization with mercuric chloride for a period of eight minutes gave satisfactory results. After sterilization in a solution of mercuric chloride, buds of all plants were carefully washed in several portions of sterile water for five to eight minutes each and they were transferred in conditions of a sterile room to the nutrient medium in test tubes 22 × 200 mm containing 20 ml of medium.

The nutrient mediums were prepared according to the directions of White [1], Gautheret [12] or Heller [13], with the addition to them of a mixture of trace elements according to Heller: 2% sucrose or 2.5% glucose and 0.8% agar.

The following groups of physiologically active substances were added in various combinations according to the conditions and objectives of the experiment to the basic nutrient medium:

I. Vitamins: thiamine (0.1 mg/liter), nicotinic acid (0.5 mg/liter), pyridoxine (0.1 mg/liter), mesoinositol (0.5 mg/liter), choline (0.1 mg/liter), riboflavin (0.05 mg/liter), folic acid (0.1 mg/liter), carotene (0.5 mg/liter), calcium pantothenate (0.8 mg/liter), and biotin (0.005 mg/liter).

II. Growth stimulants: β-indoleacetic acid (IAA), usually in a concentration of 0.05 mg/liter; gibberellic acid (GA), 1-0.5 mg/liter.

III. Purine and pyrimidine bases, their analogs, derivatives and antimetabolites: adenine, cytosine, uracil, kinetin, adenosine, guanosine, uridine, and cytidine. Usually the substances were added to the medium in a 1 mg/liter concentration, but in individual cases kinetin was added in a 2 mg/liter concentration. If a mix-

ture of nucleoside: RNA (adenosine, guanosine, cytidine, uridine) was added to the medium, their usual concentration was 2 mg/liter. As antimetabolites of the nuclein metabolism, we added 8-azaguanine or 10 mg/ /liter of ribonuclease to the medium.

IV. Substances active in regulation of the growth and development of plants: triiodobenzoic acid (1 mg/ /liter) or maleic hydrazide (MH) (5-8 mg/liter).

The nutrient mediums were sterilized in an autoclave at a pressure of 0.75 atm for a period of 20 min. All physiologically active substances were introduced into the nutrient medium prior to putting it in the autoclave because according to the available data they are thermostable. Antibiotics filtered through a Zeiss bacterial filter were added to the medium after being autoclaved. The isolated terminal buds were grown in the conditioning greenhouse of the Artificial Climate Station. The temperature in the greenhouse was 22-24° and the relative humidity about 60%. Cultures of the isolated tips were grown in conditions of a long 16-hour day, on a short nine-hour day and in continuous darkness. In the autumn, winter and early spring months the natural short day was lengthened by lighting with fluorescent lamps and incandescent lamps hung 50 cm over the plants. The short day and continuous darkness were created by placing the cultures under dark photoperiod chambers for 15 hours per day or correspondingly for the entire time of the experiment. Experiments on growing isolated buds were carried out five times with perilla, five times with rudbeckia, three times with tobacco and one time with soy and lupine.

EXPERIMENTAL RESULTS

Microscopic investigation of the buds at the time of isolation showed that the very tip of the main shoot of vegetative plants consisting of the growth cone with leaf tubercles and two to three rudimentary leaves covering the cone is transferred to the artificial nutrient medium. Promeristem tissue of the axillary buds is seen in the axilla of these leaves. The apexes of plants used in the isolated culture differed in the form of the growth cone by the number of axillary buds and the degree of their development and by other features.

Terminal buds of all plants used in the experiment (except blue lupine, which, having developed several leaves, stopped growth and died) grew well on the artificial nutrient mediums and formed 8-10 pairs of leaves, lateral shoots and a root system. Tobacco plants failed to start flowering in the culture conditions, but the miniature perilla, rudbeckia and soy plants formed reproductive organs and flowered in the test culture under corresponding conditions, and perilla in some cases gave well-developed seeds from which plants that were normal in external appearance were grown. Mamont tobacco, rudbeckia and red perilla plants grown on the artificial medium are presented in the photographs (Figs. 1 and 2). An external view of buds at the time of isolation and placement on the nutrient medium is shown above them.

The Effect of Conditions of Nutrition and Cultivation on the Growth and Organogenesis of Isolated Terminals

The nutrient mediums of White, Gautheret and Heller served as the best substrate for growing terminal buds for all of the plants being studied. However, according to our observations, red perilla buds grew best on the Gautheret medium, but tobacco buds grew best on the White medium. Substitution of sucrose for glucose in the nutrient medium did not affect the growth and development of the terminal buds, but a decrease in the concentration of sucrose in the medium from 2-5% to 0.5% significantly retarded growth. The application to

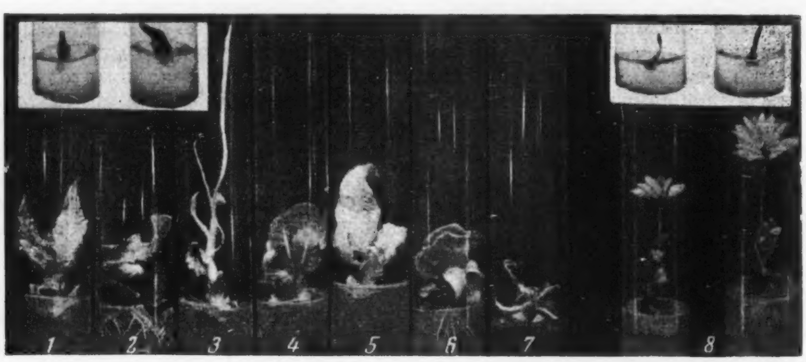

Fig. 1. Mamont tobacco (1-7) and rudbeckia (8) plants grown from terminal shoots on an artificial nutrient medium. Tobacco grown on long day (LD); 1) control; 2) medium with IAA; 3) with GA; 4) with adenine; 5) with kinetin; 6) with an extract from tobacco grown on long day; 7) same on short day; 8) rudbeckia on short day on medium with GA.

the basic nutrient medium, containing mineral salts, trace elements and sugars, of one of the groups of physiologically active substances was not necessary for the accomplishment of all processes of growth and development of the isolated buds in culture conditions, but the addition of the latter to the medium significantly changed the character of the physiological activity of the terminal meristems, and consequently the entire path of growth and morphogenesis of the terminal bud. Features of the action of these active substances will be examined below.

The age of the plant from which the bud was taken and, when axillary buds were used, the level of the leaf from the axilla of which the bud was isolated showed a great effect on the character of the growth of the terminal bud. The less the absolute age of the plant or the higher the level of the leaf and, under substantially equal conditions, the more intensively the buds grew, the earlier they developed roots and a stem and the later they began formation of reproductive organs.

The growth of the isolated bud on the nutrient medium began four to five days after planting. First the covering leaves on the bud branched out and grew; then some stem growth and formation of new leaves began; after 12-15 days callus formed on the end of the shoot submerged in the medium, and then with favorable conditions the callus developed roots at the beginning. There was a correlation between the formation of roots and the intensity of growth of the shoot; the stem began to grow rapidly only after the appearance of roots. The buds taken from relatively old plants or plants growing on mediums with gibberellic acid and kinetin often did not form roots and did not develop a rapidly growing main shoot. However, for soy, perilla and rudbeckia plants, the absence of roots and the insignificant growth of the stem did not serve as an obstacle to flowering of the developing miniature plants (Fig. 1, 8 and Fig. 2, 6 and 7).

The light regime of the cultivation of isolated buds significantly changed the character of their growth and development and also affected the effect of the physiologically active substances applied in the nutrient medium.

Features of the Action of Physiologically Active Substances Applied in the Nutrient Medium

The basic idea behind the use of different groups of physiologically active substances for the action on the terminal meristems was the importance of the intensity and specificity of the nucleic metabolism of the meristem for the process of its transfer into generative condition. For a study of this process, purine and pyrimidine bases and their derivatives, which from our point of view would increase the intensity of the nucleic and protein metabolism of the meristem, or such antimetabolites of this metabolism as 8-azaguanine or the enzyme ribonuclease were added to the nutrient medium. Heteroauxin (β-indoleacetic acid) and gibberellic acid were used as active substances opposite in nucleic derivative based on their action on the process of growth. It was also interesting to study the action of MH and triiodobenzoic acid—substances having a definite relationship to the nucleic and auxin metabolism of plants [14,15,16].

Purines, Pyrimidines and Their Derivatives

Substances of the purine and pyrimidine groups show the most positive effect on the growth and organogenesis of isolated terminals. Purines and pyrimidines applied in the medium caused, as a rule, the formation of a heavy callus on the parts of the stem submerged in the medium. On the long day numerous slowly growing rudiments of roots appeared. On the short day and in conditions of continuous darkness, the callus completely failed to form roots. Substances of this group caused

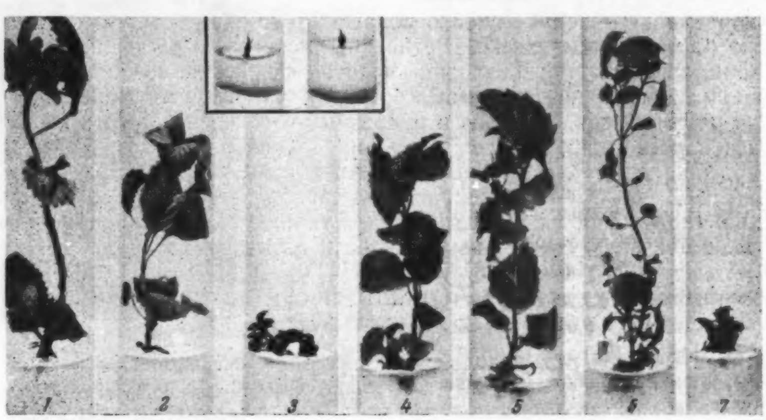

Fig. 2. Perilla plants grown on long day. 1) Control; 2) with IAA; 3) with GA; 4) with adenine; 5) with kinetin; 6 and 7) flowering with adenosine; 6) plant developed roots; 7) without roots.

some inhibition of the apical growth of the stem and a stimulation of growth of all of the axillary buds. The action of kinetin is especially characteristic in this connection. Two Mamont tobacco plants grown in the dark on the control medium and on the medium with kinetin are shown in Fig. 3. While the first plants are characterized by hypertrophic elongation of the stem, the plants from the medium with kinetin develop a clearly expressed "bushlike" form as a result of the removal of apical dominance and stimulation of the growth of all apillary buds. Rudbeckia plants on the medium with purines and pyrimidines were characterized by a tendency toward the formation of a rosette form even in long day conditions, usually causing stem growth for Rudbeckia. Purines and pyrimidines also accelerated the establishment of the reproductive organs.

Differentiation of the flower rudiments for red perilla on the long day, which is unfavorable for flowering, under the effect of adenine and kinetin in the medium, was described in an article by M. Kh. Chailakhyan and the author [17]. The effect of purines and pyrimidines, and also their analogs and derivatives, on the generative development of isolated terminal buds of red perilla was described and discussed by the author in his article in collaboration with M. Kh. Chailakhyan and I. I. Lyubarskaya (being published). For this reason this question is not discussed in detail in the present article.

β - Indoleacetic Acid (IAA)

As a rule IAA applied in the medium in an optimal concentration of 0.05-0.1 mg/liter caused for all plants in the isolated culture a more rapid and abundant development of the root system and an increase in the growth of the shoot in length. However, when perilla buds were cultivated under conditions of continuous darkness, IAA usually led to an inhibition of the growth of plants, obviously as a result of the increase in the concentration of auxin in the tissues from the tip. Under short-day conditions on the medium with IAA, perilla flowered earlier and more abundantly than in the control variant.

Gibberellic Acid (GA)

Gibberellic acid inhibited the formation of callus and a root system for isolated terminals of soy, perilla, rudbeckia, and tobacco and caused a sharp elongation of the stem and petioles of leaves of Mamont tobacco, Kharbin soy and rudbeckia, but for red perilla, on the other hand, it somewhat suppressed the growth of the stem portion. Gibberellic acid caused a strong retardation of stem growth for all plants in all cases in the dark. For tobacco plants, soy and rudbeckia, the leaves on the medium with gibberellic acid had a chlorotic appearance. Perilla plants on the short day, which is favorable for flowering for this plant, flowered on the medium with gibberellic acid earlier than in all of the remaining experimental variants. Rudbeckia flowered on the nine-hour day, which is unfavorable for its development, on the medium with gibberellic acid (Fig. 1).

Vitamins

The effect of individual vitamins on the growth and organogenesis of isolated terminals was not studied. The application in the medium of a mixture of three vitamins (thiamine, pyridoxine, nicotinic acid) or of ten vitamins (see list in description of method) stimulated growth in the case of tobaccos, but retarded growth for perilla; however, the mixture of vitamins did not cause sharp changes in the character of the growth and organogenesis of the isolated buds of any of the plants that were used.

Triiodobenzoic Acid and MH

Triiodobenzoic acid and MH added to the medium in a concentration of 8-10 mg/liter caused a sharp inhibition of the growth of the isolated terminals, and then their quick death. Obviously, the concentrations that were used were too strong. Only when isolated perilla buds were cultivated in continuous dark did the joint addition to the medium of 1 mg/liter of kinetin lead to an acceleration of flowering of the tips as compared to the control grown under the same conditions.

DISCUSSION OF RESULTS

As the experiments that were carried out showed, culturing terminal meristems in vitro makes it possible to affect more easily than for the entire plant the path of the physiological processes in the meristem and control them with the help of chemical factors. This is determined by the fact that the terminal bud, which still has not formed roots and leaves to any significant

Fig. 3. Removal of the apical dominance for tobacco plants on a medium with kinetin. 1) Medium with kinetin; 2) control (dark).

degree, depends for its activity in the first period after its isolation on the composition of the nutrient medium and substances of high physiological activity applied in it. Such "plasticity" of the meristem and the possibility of controlling the process of plasmatic chemodifferentiation of its cells, and also the path of growth and organogenesis of the developing plant stemming from this, make the method of culture of isolated buds very attractive for the study of the physiological features of the meristem by shifting the meristem into a generative condition controlled either by the conditions of cultivation or by the action of chemical factors.

SUMMARY

1. Conditions of cultivation were developed in a sterile culture on artificial nutrient mediums for isolated terminal buds of mature vegetative plants of red perilla, rudbeckia, Kharbin soy and two species of tobacco.

2. The terminal buds isolated from the plant in culture conditions on nutrient mediums containing mineral elements and sugar grew, developed lateral shoots and eight to ten pairs of leaves, and developed roots; and miniature plants of soy, perilla and rudbeckia budded out, flowered and developed seeds in certain conditions.

3. Both the age of the plant from which the bud was taken and the position of the bud on the plant affected the process of growth and organogenesis of the isolated bud. Buds from young plants or from the axilla of leaves of the upper level grew better and formed roots more easily. Buds from old plants or from the axilla of leaves of the lower levels shifted into flowering more rapidly.

4. The conditions of cultivation (light regime) and also the addition to the medium of physiologically active substances of the type of auxins, purines and pyrimidines, and vitamins significantly changed the intensity and character of the growth and organogenesis of the isolated bud.

5. Substances of a purine and pyrimidine nature (bases, nucleosides and kinetin) caused some retardation of the growth of the roots and the main shoot, but significantly stimulated the meristematic activity of all of the axillary points, and also stimulated the generative differentiation of the points of growth of soy and perilla plants, both on a favorable and on an unfavorable photoperiodic regime.

In conclusion, the author extends his thanks to Professor M. Kh. Chailakhyan for his concern with this work and for his valuable suggestions, and also to laboratory workers Z. M. Yakovleva and I. I. Lyubarskaya for help in carrying out the difficult and precise work connected with the culturing of a large number of isolated terminal buds.

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THE EFFECT OF COBALT ON THE GROWTH AND DEVELOPMENT OF COTTON

Z. I. Mamedov

Ministry of Agriculture, Azerbaijan SSR, Baku

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The investigations of many authors [1-4] have established that such trace elements as boron, manganese, copper, molybdenum, zinc, iodine, iron and others are necessary for plant life.

In addition, there are many studies showing that cobalt is highly effective in increasing the yield of a number of agricultural crops: clover, flax, bean, mustard, barley, oats, eggplant, cotton and other plants.

We studied [7] the effect of cobalt on the development, yield, and on several physiological processes of cotton in the conditions of Azerbaijan.

Experiments with the application of cobalt under cotton (variety 1298) were carried out on the Lenin collective farm in Mardakersk region, Kalinin collective farm in Saatlinsk region, and on the Mugansk Zonal Experiment Station, under field conditions with three repetitions; each plot was 300 m^2 in size. The effect of cobalt on the development, yield, and on several physiological processes was studied against a background of nitrogen-phosphorus fertilizers. Cobalt was applied to the soil prior to planting and during the periods of bud formation, and it was also applied by foliar methods.

The results of our experiments show that when cobalt is applied to cotton, the growth of the plants is significantly increased, the number of bolls is increased, and the dropping of the bolls is decreased (Table 1). The greatest effect of the application of cobalt is obtained on saliferous soils.

We determined the normal water content of the leaves during the different hours of the day (Table 2) in order to show the positive effect of cobalt on the growth and development of plants.

Table 2 shows that the concentration of water in the leaves of plants receiving cobalt somewhat exceeds the concentration of water in control plants.

The concentration of the cell sap was also studied in the experiments that were carried out. The results of the investigation are presented in Table 3.

The data in Table 3 show that a noticeable decrease in the concentration of the cell sap in the cotton leaves takes place under the effect of cobalt, especially at 1 P.M. It is necessary to note here that the soaking of seeds in a 0.025% solution of $CoSO_4$ (Mardakersk region) approaches the level of the variant where cobalt was applied in the soil at the rate of 5 kg/hectare.

In the experiments that were carried out, we also studied the effect of cobalt on the activity of the oxidation-reduction processes of enzymes and, in particular, on the activity of catalase.

The positive effect of cobalt on the activity of catalase in cotton leaves was established by experiments in 1957. Samples were taken on July 28, 1957, at 9 A. M. from the seventh and eighth levels, counting from the bottom. The data that were obtained are presented in Table 4.

TABLE 1. The Effect of Cobalt on the Growth and Development of Cotton

factors considered	1957 experiment with soaked seeds		1957 expt, with cobalt applied to the soil			
			nonsaliferous		saliferous	
	in water	in 0.025% solution of $Co(NO_3)_2$	control	$Co(NO_3)_2$, 1 kg/ha	control	$Co(NO_3)_2$, 1 kg/ha
growth of plants, cm	62.2	90.6	81	110	31	55.8
number of bolls formed	9.3	16.3	16.1	25.2	4.2	11.8
number of bolls dropped	10.2	4.2	48.6	26.4	21.8	8.2

TABLE 2. The Effect of Cobalt Nitrate on the Water Content of Cotton during Different Times of Day

time of taking samples for analysis	Mardakersk region, Sept.10, 1956				Saatlinsk region, Aug.21, 1957			
		$Co(NO_3)_2$			saliferous soil		nonsaliferous soil	
	control	in soil, 5 kg/ha	in soil, 2 kg/ha	sprayed, 0.025% solution	control	$Co(NO_3)_2$ in soil, 1 kg/ha	control	$Co(NO_3)_2$ in soil, 1 kg/ha
7 A. M.	71.7	72.5	71.1	73.4	77.1	77.8	79.0	79.4
1 P. M.	69.6	71.1	70.8	71.4	75.5	77.3	73.5	75.5
7 P. M.	70.6	73.5	71.7	71.6	76.2	77.91	76.5	77.5

TABLE 3. The Effect of Cobalt Sulfate on the Concentration of the Cell Sap in Cotton Leaves at Various Times of the Day

time of taking samples of analysis	Mardakersk region, Aug. 29, 1956				Saatlinsk region, July 11, 1957	
	control	in soil, 5 kg/ha	in soil, 2 kg/ha	soaking seeds in 0.025% solution	control	in soil, 1 kg/ha
7 A. M.	13.67	12.16	12.57	13.57	7.78	8.28
1 P. M.	18.78	16.09	14.66	16.28	10.88	9.08
7 P. M.	14.8	14.08	13.48	14.53	8.50	7.5

TABLE 4. The Effect of Cobalt on the Activity of Catalase in Cotton Leaves

experimental variant	quantity of oxygen given off, mg			
	after 3 minutes	after 6 minutes	after 9 minutes	after 12 minutes
control	61.2	70.8	70.8	70.8
application of 1 kg $CoSO_4$/ha	64.6	72.6	72.7	72.8

We can see that some increase in the activity of catalase takes place, particularly after the first three minutes, under the effect of cobalt.

Supplying the demands of cotton for cobalt significantly accelerates the development of the plants and decreases the dropping of bolls, thus increasing the yield of cotton.

Thus, on the Lenin collective farm in Mardakersk region, a yield of cotton of 23 centners per hectare was obtained without the application of cobalt (control) in 1956, but the yield of cotton was increased to 25.1 centners per hectare, or 109% of the control, with the application of 5 kg per hectare of cobalt to the soil; the yield was increased to as much as 26.9 centners per hectare (or 116%) with the application of 2 kg per hectare, and when the seeds were soaked in a 0.025% solution of $CoSO_4$, the yield was 26.2 centners per hectare or 113.9% of the control.

A yield of cotton of 15.21 centners per hectare was obtained in 1957 on the Kalinin collective farm in Saatlinsk region on saliferous soils without the application of cobalt (control), but when cobalt sulfate was applied to the soil at the rate of 1 kg per hectare, the yield was 18.47 centners per hectare (increase of 21%); the yield of cotton on nonsaliferous soil was correspondingly 25.9 and 30.62 centners per hectare (increase of 18%). In 1958 the yield was 5.85 centners per hectare on saliniferous soils on this same collective farm without the application of cobalt, and it was 6.83 centners per hectare when 1 kg of $CoSO_4$ per hectare was applied (increase of 17%).

The data that were obtained show that: 1) cobalt gives the best results when applied under cotton plants prior to planting; 2) application of cobalt accelerates the growth and development of cotton plants; 3) application of cobalt in the soil significantly increases the concentration of water, decreases the concentration of cell sap and strengthens the activity of catalase in cotton leaves; 4) cobalt significantly increases the formation of bolls, decreases their dropping by 22.2%, and improves the yield of cotton by about 9-21%.

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THE EFFECT OF ARTIFICIAL ILLUMINATION ON PHOTOSYNTHESIS AND THE REPRODUCTIVE DEVELOPMENT OF PLANTS

G. A. Odumanova

All-Union Institute of Plant Protection, Leningrad
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The use of artificial illumination in the cultivation of plants is becoming increasingly more important both for farming and for research purposes.

Artificial light permits a considerable shortening of the growth period for many crops and can increase their yield. By using incandescent lamps, Moshkov [1] obtained a crop of ripe tomatoes in 40-45 days. Under continuous lighting from these lamps, branching wheat was forced to maturity in 70 days with a seed yield of 3-4.5 g per spike. The cultivation of seedlings under electric light is receiving widespread application.

In a number of ways, however, artificial light is inferior to natural light. This is especially true of the intensity of illumination.

In the present work the effects of light intensity on photosynthesis and on the photoperiodic reaction of plants were investigated. Experiments were carried out with oil perilla in the laboratory of light physiology under conditions of artificial illumination. Photosynthesis was determined according to the method described by V. A. Chesnokov and E. N. Bazyrina. The leaf chambers were described by us earlier [2].

On perilla plants about a month old it was maintained for one leaf (of the fourth stage) and for two axillary shoots (of the third stage). For a solution of the given problem a number of operations were carried out with the experimental leaves. They amounted to varying the conditions of light intensity received by the leaves and consisted in the following: With the help of lightproof black paper one side of the leaf was completely deprived of light. A piece of this paper somewhat larger than the leaf surface was sewn onto the blade of the leaf with large neat stitches of white thread. A total of four treatments was set up (Fig. 1).

The experimental plants were placed in an apparatus with 500-w incandescent lamps (reflecting) with water filters. The side of the leaf which was turned upward received light with an output of 150-220 w/m^2 and an illumination of 11,000-12,600 luxes; on the side of the leaf which was turned downward there fell mostly a reflected and diffuse stream of light with an output of 6-7.5 w/m^2 and an illumination of 320-370 luxes. For 20 days (May 9 to 29, 1956) all the variants were given a short eight-hour day. At the end of this period all the leaves were removed from the black paper and placed under conditions of continuous illumination in the same light apparatus.

Literature data on the effect of light intensity on leaf activity are almost nonexistent. In his short-term experiments Bussengo [3] ascertained that both sides of the leaf are capable of fixing carbon dioxide.

Analysis of the numerical data on the rate of photosynthesis covering the whole range of photoperiodic activity which we studied showed the following (Table 1). Although the light in our apparatus was of low intensity, since on the lower side the leaf received a light beam which was weakened by more than 20 times, the elimination of the light falling on the lower side of the leaf still led to a considerable decrease in photosynthesis. Photosynthesis in the control treatment averaged 3.61 mg CO_2/dm^2 per hour, while photosynthesis in the leaves deprived of light below (treatments 3 and 4) amounted to only 2.16-2.22 mg CO_2/dm^2 per hour, i. e., was almost 40% less.

When the overhead light was eliminated the assimilation of carbon dioxide by the lower side of the leaf was extremely weak (treatment 2). How can such a result be explained: Does the reduced illumination alone have an effect or does the lower side of the leaf have, in general, a reduced capacity for assimilating carbon dioxide? It would seem that the whole thing has to do with the illumination, since the potential of the lower side of the leaf is sufficiently great. This was determined with the help of treatment 4. Under one-sided illumination from above, the lower side of a leaf which was turned upward carried on photosynthesis equal in magnitude to photosynthesis of the upper side of the leaf (treatment 3).

These circumstances would indicate that the light apparatus with incandescent lamps produces a light beam which, because of its low intensity, permits the realization of only a small part of the considerable potential for photosynthesis possessed by the lower side of the leaves. This brings about a reduction in the assimilation of carbon dioxide.

TABLE 1. Rate of Photosynthesis in Perilla in the Different Treatments of the Experiment

Date	Leaf area, cm^2	Leaf temperature, °C	CO_2 concentration, mg/liter	Photosynthesis mg $CO_2/dm^2/hr$
Treatment 1; leaf area 172.5 cm^2				
14		20 —22	0.717	4.25
16		18.8—19.8	0.643	2.76
17		20 —22	0.717	3.57
18		20 —21.4	0.727	3.80
22		21.8	0.752	4.02
24		21 —21.5	0.677	3.89
26		22 —23.2	0.707	2.95
				av. 3.61
Treatment 2; leaf area 120.7 cm^2				
15		25 —25.6	0.680	0.14
16		23 —24.8	0.643	0.63
17		21.6—23	0.717	0.24
19		23.3—24	0.687	0.05
22		22.4	0.752	0.34
24		22 5—23.8	0.667	0.14
				av. 0.26
Treatment 3; leaf area 190.7 cm^2				
14		24 —25	0.717	2.29
15		23.2—25	0.680	1.86
17		23.6—24.4	0.717	3.05
18		19.2—22.4	0.727	2.35
19		24 —26	0.687	2.05
24		24.8—26.2	0.677	1.50
26		24.5—25	0.707	2 47
				av. 2.22
Treatment 4; leaf area 159.7 cm^2				
14		24 —25	0.717	2.33
15		27 —27.6	0.680	1.35
16		21 —22.8	0.643	2.19
18		23 —24.2	0.727	1.53
19		25.2—26	0.687	2.74
22		22.3—23.4	0.752	2.83
26		24 —26.1	0.707	2.15
				av. 2.16

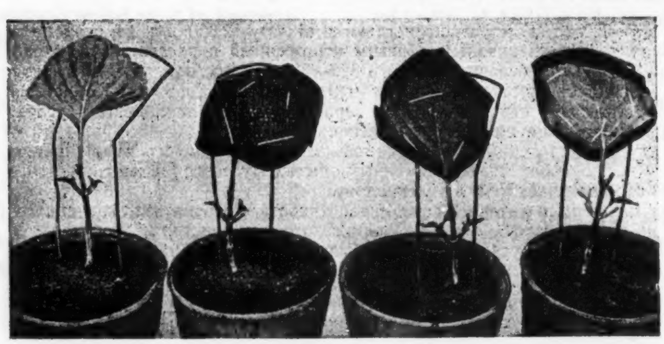

Fig. 1. Treatments in the experiment with white light. 1) Control, leaf not covered; 2) upper side of the leaf covered with black paper with the edges folded underneath; the paper was covered with tracing paper; the lower side of the leaf was left uncovered and turned downward; 3) lower side of the leaf covered with black paper with the edges folded upward; the upper side of the leaf was left uncovered; 4) upper side covered with black paper and turned down; the edges of the paper were folded upward; the lower side of the leaf was left uncovered and turned upward.

If we follow the performance of the upper and lower sides separately in the normal position of the leaf (treatments 2 and 3) then we find that the sum of the assimilation activity of the sides does not come close to attaining the rate of photosynthesis in the control leaf. The difference is about 30%. This indicates that the one-sided low-intensity illumination brings about a considerable decrease in the photosynthetic activity of the leaf. It is probable that a similar decrease occurs both on account of respiration of the darkened side and due to the reduced activity of the unilaterally illuminated side of the leaf.

The low values for the rate of photosynthesis even in the treatments with illumination 1.5-2 times higher than the saturation point could be explained as a peculiarity of the photosynthetic apparatus under conditions of artificial illumination. Chesnokov and Stepanova [4] have pointed out similar facts.

At the end of the 20-day photoperiodic stimulus an anatomical analysis of cross sections of the leaf was carried out on the various treatments of the experiment. The sections, obtained with the help of a microtome, were 6 μ thick. The data are given in Fig. 2. When the light was excluded the whole light beam was absorbed by the leaf through its dorsal side (treatment 3). This made little change in the structure of the spongy parenchyma, whereas it abruptly lengthened the cells of the palisade parenchyma of the leaf. Treatments 2 and 4 were placed under very unusual conditions: In them the upper side of the leaf was deprived of light and all of the light beam received by the leaf was directed onto its lower side, i.e., onto the spongy parenchyma. The anatomical changes in the tissue were then serious. Functions which are usually characteristic for the palisade parenchyma fell to the lot of the spongy parenchyma. And we see how the spongy parenchyma was adapted for this purpose; its cells greatly increased in size and were arranged vertically in more or less regular rows, and their number was considerably reduced. The palisade parenchyma was no longer as clearly expressed as in the control leaf. Thus the difference between the palisade and spongy parenchyma was somewhat reduced. The treatments in this experiment (2 and 4) differed only in the intensity of light falling on the lower side of the leaf. And correspondingly, the thickness of the more poorly illuminated leaf (treatment 2) was found to be less in comparison with the better illuminated leaf of treatment 4. Thus conditions of illumination could be detected not only by its activity but also by the anatomical structure.

In the experiment described the intensity of the light falling on the leaf from above was 2-3 times greater than the saturation point, i. e., the intensity which permitted the maximal reduction of the period of development. Under these conditions the light intensity had little effect on the nature of the photoperiodic reaction. This was true for both the development period and the extent of flowering. However, this occurred only in those cases when the dorsal side of the leaf was turned toward the light. If the lower side was exposed to the light (treatment 4) then, regardless of the equality of the light, the photoperiodic reaction was found to be somewhat more weakly expressed. This was shown by the retardation of flowering and by the decrease in its extent (Table 3).

The data presented very obviously show that if both sides of a leaf are capable of photosynthesis at the same rate, then the dorsal side possesses some advantages with respect to photoperiodic reaction. If we compare the data in the first and second tables, then we will be convinced that the decreased assimilation brings about a very late and weak reproductive development (treatment 2). Here there is a very close dependence of photoperiodic reaction upon the rate of photo-

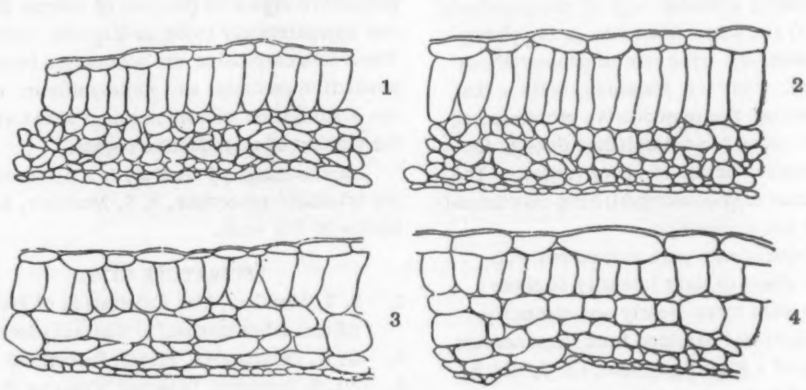

Fig. 2. Effect of conditions of illumination on the anatomical structure of the leaf of perilla; 1,2,3,4) number of the treatment in the experiment; explanation in the text.

TABLE 2.

Date (June)	Leaf area, cm^2	Average leaf temp., °C	CO_2 concentration in air, mg per liter	Photosynthesis mg CO_2 per dm^2 per hr	Date (June)	Leaf area, cm^2	Average leaf temp., °C	CO_2 concentration in air, mg per liter	Photosynthesis, mg CO_2 per dm^2 per hr
Red-orange light 60 $w/m^2 \times 2$					Red-orange light 60 w/m^2				
13	34.2	20.4	0.645	4.08	13	34.5	22.7	0.645	0.84
14	34.2	21.5	0.653	2.49	14	34.5	21.9	0.653	—0.51
15	34.2	23.8	0.723	1.70	15	34.5	24.1	0.723	2.36
16	34.2	22.0	0.575	1.19	16	34.5	22.0	0.575	—1.18
21	38.2	23.9	0.713	2.59	21	38.2	21.1	0.713	0.00
22	38.2	23.2	0.700	4.57	22	38.2	21.8	0.700	—0.99
23	38.2	23.7	0.633	3.81	23	38.2	21.6	0.633	—0.16

TABLE 3. Development of Reproductive Organs in Perilla in the Different Treatments of the Experiment

treatment No.	number of days before laying of inflorescence initials*	number of days before flowering*	number of inflorescence initials	number of reproductive organs†
1	25	33	23	282
2	43	–	5	16(buds)
3	25	33	22	243
4	25	41	15	136

* Number of days before laying of inflorescence initials and before flowering was counted from the beginning of photoperiodic stimulus.

† Number of reproductive organs was the average count per plant.

synthesis. By the attainment of some optimal values of photosynthesis this dependence then fades into the background, for here we see the same reproductive development under significantly different rates of photosynthesis (treatments 1 and 3) and some difference in the photoperiodic reaction under the same rate of photosynthesis (treatments 3 and 4). The facts presented indicate that the connection between the reproductive processes and the assimilation of carbon is accomplished through the control of the qualitative nature of photosynthesis. The quantitative properties of photosynthesis have only secondary importance in this connection.

A number of experiments were carried out with colored light. The effect of light intensity on photosynthesis was shown even more clearly and distinctly here. Red-orange light was obtained from incandescent lamps with the help of a glass light filter KS-5, which allows the passage of radiation starting at 600 mμ. Special chambers for the leaf, with a water shield and ventilation [5], where the leaf was illuminated from one or two sides, were equipped with such light filters.

The results of the investigations showed that under two-sided illumination with red-orange light the gas exchange had a normal, firmly positive nature (Table 2). Under one-sided red-orange illumination the leaf was found to be under conditions of almost complete absence of light, or the red-orange light was to a considerable extent absorbed in the this surface layer of cells [6], leaving only the infrared part of the spectrum for the deeper-lying layers. In this case a sharp reduction was found in the apparent assimilation in the leaf. A similar phenomenon was also noted by Voblikova [7]. If we introduce a tentative average correction for respiration for both treatments (according to our measurements it averaged 1.67 mg CO_2/dm^2 per hour for 60 $w/m^2 \times 2$, and 2.56 mg CO_2/dm^2 per hour for 60 w/m^2) then we will easily be convinced that even then photosynthesis under intense illumination will be much higher. Consequently, under conditions of unilateral red-orange illumination the organic end result is not just due to an increase in the oxidation processes, but a regular decrease of the photosynthetic activity of the leaf also occurs.

The inflorescence initials were laid at the same time in both treatments. However, the number of reproductive organs in the case of intense illumination was approximately twice as large (36 compared with 16). These data emphasize the connection between the reproductive processes and photosynthesis: A more vigorous assimilation of carbon brings about an increase in the number of reproductive organs.

In conclusion, I consider it a pleasant duty to thank my scientific supervisor, B. S. Moshkov, for his valuable advice in this work.

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THE LARGE DEVELOPMENT CYCLE OF THE POTATO PLANT

Yu. G. Trinkler

M. Gor'kii Pedagogical Institute, Gor'kii
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Generally the potato grows as an annual plant; its leaves complete their development annually, then die in the autumn. However, the potato overwinters in the form of tubers, which are a vegetative part of the plant. Therefore, it is more correct to consider it as a polycarpic plant with monocyclic shoots.

The most general rule of ontogeny for all polycarpic plants, first noted by I. V. Michurin amd further developed by Soviet scientists [1,2,3] and others, is the presence of two cycles of development: the large cycle (from the development of the seeds up to maturity and natural death of the plant) and the small cycles of development (annual development of individual shoots). Consequently, one can consider that potato, as a polycarpic plant, has large and small cycles of development. It is clear that the features of the development of the potato in the small cycle, including yield and other agriculturally important qualities, will differ according to the stage of the development of the large cycle. In particular, plants in the stage of old age of the large cycle will be diseased plants. These ideas correspond to the theory of the aging of varieties. This theory was considered only orally, in spite of its great age. The investigations that were carried out also had the goal of experimental verification of the indicated ideas.

For potato, progressive aging can take place in proportion to the portion of the stem between the planted tuber and the stolons. While this aging is practically unnoticeable for one generation, these age changes can be very significant after ten years. The nature of the method of accelerating the development of potato in the large cycle is included in the fact that the length of the stem from the planted tuber to the new tuber was increased approximately to 20 nodes. This was accomplished by means of grafting tops of stems and obtaining tubers from the grafts. The experiments were carried out with seedlings as young plants. Insofar as the plants of all variants were vegetative descendants of the original seedling, all were representatives of one clone. Because each following variant of this experiment was biologically older than the previous one, the combination of experimental variants was called a ladder clone (figure). Each variant of the ladder clone was called a "degree." The tuber generations of the original seedling were degree I. In the year of planting or in the following year, tip grafts were taken from the seedlings. Tubers developed on them and produced the beginnings of plants of degree II. Plants of degree III were obtained from these plants by the same method, and then IV, V and VI. As an indication of the biological age, morphological features, usually the number of nodes formed by stems different degrees from the cotyledons up to the given graft, were used. The first four ladder clones were formed in 1949 from seedlings of varieties of Smyslovskii (Table 1, A, B) and variety Regina (Table 1, C, D). Later experiments were started with seedlings of varieties of Épron (Table 1, E, F), Smyslovskii (G) and Majestic (H). Tests were carried out over a period of six years (degree I was cultivated for the period of the entire experiment for comparison). Data on the yield are presented in Table 1. The six-year tests were completed for seven clones for degrees I and II; there are data for a fewer number of years for the remaining degrees. The same experiments were carried out on plants

TABLE 1. Comparison of Average Figures of the Yield of Different Degrees of Ladder Clones from Seedlings (in grams per bush)

Degree	Approx. No. of nodes	Ladder clones								Av. per clone per year	
		A	B	C	D	E	F	G	H	g	%
I	1	382	655	495	558	669	369	687	409	532	100.0
II	20	402	762	646	789	326	355	688	454	553	103.9
III	40	451	665	330	487	78	157	147	397	352	66.2
IV	60	358	552	136	528	55	2	4	49	278	50.2
V	80	383	460	159	150	—	—	—	—	330	62.0
VI	100	185	386	19	—	—	—	—	—	240	45.1

TABLE 2. Comparison of Average Figures of the Yield of Different Degrees of Ladder Clones of Common Varieties (in grams per bush)

Degree	Approx. No. of nodes	Varieties										Av. per clone per year	
		Regina I	Smyslovskii I	Smyslovskii II	Lorkh I	Lorkh II	Rozovyi fr. Milet	Épron I	Épron II	Varba	Regina II	grams	%
I	1	1081	1153	1135	855	713	509	425	493	727	1038	813	100.0
II	20	368	1339	566	810	956	385	393	529	643	211	620	76.3
III	40	179	969	393	372	502	126	99	242	299	771	431	53.0
IV	60	49	215	74	174	556	—	75	507	2	821	279	34.3
V	80	146	—	263	4	84	—	—	401	—	413	232	28.5

* We calculated the arithmetic averages from data for all clones for all years, for example, for degree VI (Table 1):

$$\frac{(116+46+81+498)}{4}+\frac{(162+353+642)}{3}+\frac{19}{1}=\frac{1917}{8}=240.$$

of the usual varieties (with ten clones of six varieties – see Table 2). The tests of each degree were carried out over a period of five years. Now there are also complete data for the first two degrees. All experiments were carried out under field conditions. In addition to the yield of tubers, we also studied other indicators of the yield and development of the plants.

Table 1 shows that, based on the yield, degree II is close to degree I. With the exception of the ladder clones E and F, the yield of degree II is equal to or higher than the yield of degree I. This leads to the fact that there is on the average a similar increase in the yield of degree II. The yield decreased in sequence for degrees of a higher order, showing the fall in the vegetative vigor of the plants of these degrees, that is, progressive aging. Complete degeneration had already occurred for degree IV for ladder clones F and G.

A somewhat different picture is shown for ladder clones of the usual varieties. Although for three clones (out of ten), degree II also shows greater yield than degree I, first, these cases are fewer, and, second, degree I on the average still has the greater yield. The following degrees also show decreased yields. With this, a sharp degeneration is observed for seven clones of degree IV. For comparison of the path of the decrease in yield for the following degrees in these two series of experiments, the average data based on them are calculated as percentages of the yield of degree I.

The results of the experiments support the idea that the yield is decreased down to complete degeneration with repeated grafting of the stem tips. As the data expressed in percentages show, this takes place slowly for seedlings (beginning of the large cycle of development) and rapidly for clones of the usual varieties (stage of old age of the large cycle of development).

The large cycle of development of potato can be divided into four stages. The juvenile stage begins after completion of the embryo stage of the large cycle from the time of germination of the seeds. Seedlings at this time have the latest flower bud formation (several seedlings in the first year did not flower at all), a large quantity of small tubers, and long stolons. The heredity of seedlings in the juvenile stage is most variable. The distinguishing traits of the seedlings are maintained for a period of four to five years. During this period (different for different seedlings) a gradual obliteration of the differences with the standard varieties, both in the date of flower bud formation and also of characteristics other than yield, takes place.

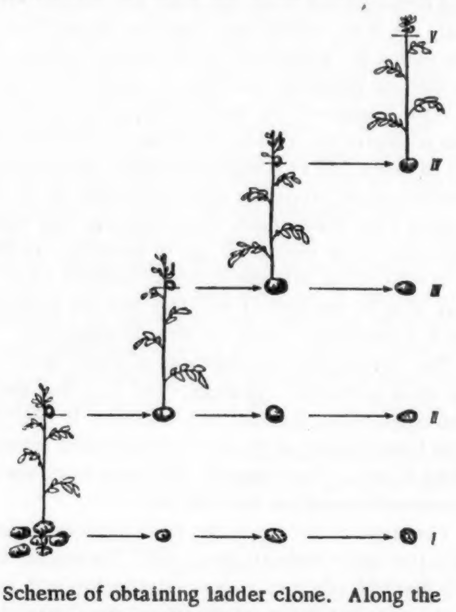
At the end of the juvenile stage, potato moves into the mature stage, that is, into the stage of the large cycle of development where the tuber descendants are usually the most vegetatively vigorous. In practice, this is the period of agricultural use of potato varieties. The data that are presented show that an increase in vegetative vigor is observed for the majority of clones for plants of degree II. This is expressed in an increased yield of tubers and by a high percentage of marketable tubers by weight, while the other indicators on the level of degree I are maintained. This indicates that with an increase in the biological age of seedlings, an obliteration of differences with the standard varieties takes place; that is, the shift of seedlings into the mature stage is accelerated, which we already noted [4].

The last stage of development of potato in the large cycle is the old-age stage. The data that are presented support this, because degeneration is obtained for the later degrees of the ladder clones with repeated grafting of tips. Consequently, the entire large cycle of potato development has been traced.

The stage of old age is the most complex stage for observation under normal conditions. The reason for this is that old age is in a close relationship with the differences in conditions of the life of the plants. Unfavorable factors (heat, drought, etc.), and also virus infections, can bring potato to degeneration, and these factors act significantly more strongly on old varieties in the stage of old age.

The complexity of the process of degeneration is still aggravated by the fact that vegetative rejuvenation takes place for the developing tubers of each bush of a given variety in different degrees. Thus, old age and degeneration take place, as was noted by Linnik [5], universally, gradually, but partially. Rejuvenation of the organs of natural vegetative propagation was noted by a number of researchers [6,7]. Linnik showed [5,8] the special rejuvenation of potato tubers, and he considered that the rejuvenation of the tubers takes place in proportion to the growth of the young cells. Although at the present time there is no generally accepted hypothesis explaining the vigor of vegetative rejuvenation, undoubtedly the vigorous flow of plastic substances to the newly formed organs (in this case, to the tubers) aids the intensification of metabolism. This, in its turn (possibly along with the following period of dormancy) probably also consists of the vigor of rejuvenation.

If there would be no rejuvenation when tubers are formed on the tip grafts, then degree II (and even more so, degree III) would have to be completely degenerated, which, however, is not observed [4]. On the other hand, the fact that a progression of old age takes place with repeated grafting of tips suggests that vegetative rejuvenation cannot completely change itself to root rejuvenation with sexual regeneration. From these ideas, it is easy to explain why ten-year potato can neither age nor degenerate. If the nature of this was such that the



Scheme of obtaining ladder clone. Along the vertical axis are the tuber generations in order; along the horizontal are the different degrees (I-IV) of the ladder clone.

tubers develop at the beginning from the lower part of the stem, then the variety would be degenerated in 10 to 15 years in proportion to the yearly buildup of old age. However, this as a rule does not happen. Consequently the reason for the absence of mass degeneration is the vegetative rejuvenation of the tubers, as a result of which the prolonged cultivation of varieties of potato is possible.

The statement of the hypothesis of ontogeny of potato makes it possible to explain the connection of the yield with the development of potato. The idea that the greatest yield is characteristic of the youngest plants is not accurate. The greatest yield is connected to the vegetative vigor of the plants. Changes in the vegetative vigor, as experiments with the ladder clones show, have in most cases the character of a monotonic curve, like the growth features according to the Krenke theory. Consequently it is a rule that seedlings, that is, plants in the juvenile stage, have lower yields than plants in the mature stage. With a shift to the stage of old age, the older the plant, the lower its yield.

The investigations that were carried out not only have theoretical interest, but can also be immediately introduced into practice. The greatest vegetative vigor of degree II seedlings can be used in the practice of seed culture for increasing the biological age of the elite by means of a single graft of tips of superelite plants.

In conformance with the indicated hypothesis, one cannot (with vegetative regeneration) fully eliminate potato degeneration, as one cannot prevent old age and death for any living organisms. One can only postpone and weaken this harmful effect by means of exact observation of seed growing rules. Therefore a change in the vegetative regeneration would be a radical method of combatting potato degeneration, along with a number of other measures. For this it is necessary to develop new varieties of potato capable of regeneration with seeds.

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THE ACTION OF GIBBERELLIN ON THE GROWTH, PRODUCTIVITY AND SHAPE OF THE FRUIT OF THE TOMATO PLANT

S. F. Negrutskii

Lugansk Agricultural Institute

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We performed special experiments at the beginning of 1958 in a greenhouse of the Lugansk Agricultural Institute to explain the effect on tomatoes of gibberellin. The investigations were made on the recommendation of the Laboratory of Plant Growth and Development of the K. A. Timiryazev Institute of Plant Physiology, Academy of Sciences of the USSR. The methodological directions for the use of gibberellin were obtained from Professor M. Kh. Chailakhyan.

Seeds of tomatoes of varieties Donets and Mayak were planted on February 18 in containers with a capacity of 6 kg, after preliminary mixing of the soil with compost (3:1). The shoots of the plants appeared on March 2. In the process of vegetation the plants were fed three times with nitrogen, phosphorus and potassium fertilizers. During the spring period the plants were grown at a temperature of 20-25°, and at the onset of warm weather the containers with the plants were placed in the open air.

For the development of our subject we used gibberellic acid (ga) from the English firm, Imperial Chemical Industries Ltd.

The treatment of the plants with the gibberellin was started when they were 15 days old. For this, the gibberellin in concentrations of 0.02%, 0.01%, 0.001%, and 0.0001% was applied daily during the morning hours with pipettes on the point of growth of the plants at the rate of one drop daily for a period of three weeks. Each variant of the experiment had five repetitions. Besides this, tomatoes grown for sprouts were subjected to the action of the gibberellin.

The test plants differed greatly from the control plants as early as ten days from the start of the gibberellin treatment.

The plants treated with gibberellin showed stronger growth, with leaves of a somewhat lighter color, and this relationship was observed only on plants treated with gibberellin in concentrations of 0.02% and 0.01%.

The difference in height between the treated and control plants was more noticeable one month from the beginning of the gibberellin treatment (Table 1, Fig. 1).

As we can see, stimulation of growth is observed for tomatoes of both varieties treated with gibberellin in the higher concentrations (0.02%, 0.01%, 0.001%) and the height of the plants is increased by 50.0-53.9% with increased concentration. The treatment with gibberellin in a concentration of 0.0001% did not cause any difference between the treated and control plants.

According to the degree of further growth of the tomatoes, the difference in their dimensions became less significant and the control plants were the same height as the plants treated with gibberellin at an age of two and a half months.

Observations of the date of flowering of both varieties of tomato showed that the opening of the flowers began as a rule one to two days earlier for plants treated with gibberellin in concentrations of 0.02% and 0.01% than for control plants.

It is interesting to note that a rapid growth of the ovary, which developed noticeably several days prior to flowering, takes place in plants treated with 0.02 and

Fig. 1 Comparative height of plants treated with gibberellin in different concentrations. 1) Control; 2) 0.001%; 3) 0.02%.

TABLE 1. The Effect of Treatment with Gibberellin on the Height of Tomatoes

Gibberellin conc., %	Donets		Mayak	
	cm	% of control	cm	% of control
0.02	21	150.0	20	153.9
0.01	19	135.8	19	146.1
0.001	16	114.3	15	115.4
0.0001	14	100.0	13	100.0
control	14	100.0	13	100.0

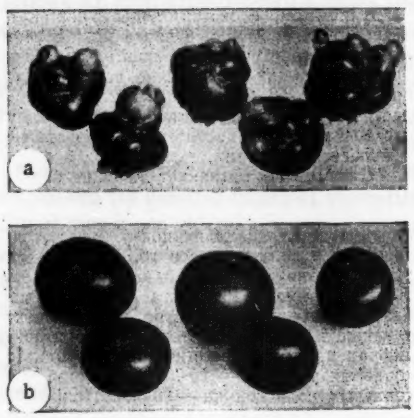

Fig. 2 The external appearance of tomatoes of the first flowers after treating plants with gibberellin. a) 0.02% and 0.01% concentrations, Donets variety; b) 0.001% concentration, Mayak variety.

0.01% concentrations of gibberellin. On the strength of this, such deliquescent flowers in a number of cases differed sharply in external appearance from the flowers of control plants.

The rate of growth of the fruits of plants treated with gibberellin was higher than that for control plants; however, the average weight of the fruit was practically the same: 54 g for tomatoes treated with gibberellin in concentrations of 0.02% and 0.01%, and 52 g for non-treated tomatoes. The ripening of the fruits appeared

TABLE 2. The Effect of Treatment with Gibberellin on the Productivity of Tomatoes

Gibberellin conc., %	Donets		Mayak	
	grams	% of con-trol	grams	% of con-trol
0.002	849	134.8	855	142.0
0.001	828	131.4	836	133.8
0.0001	710	111.1	747	124.1
0.0001	627	99.4	610	101.0
control	630	100.0	602	100.3

earlier for plants treated with gibberellin, although the final ripening took place almost simultaneously.

The calculation of the yield was made for four flowers. The data are presented in Table 2.

Analyzing the data that are presented, we find that the treatment of tomatoes with a 0.02% concentration of gibberellin leads to an increase in productivity of 34.8-42.0% compared to the control.

The treatment of plants with gibberellin in lower contrations (0.01 and 0.001%) gives a smaller increase in yield, and a 0.0001% concentration of gibberellin shows no effect on the yield of fruit (deviation from the control is within the range of the experimental error).

There is some interest in the process of fruit formation for the varieties. Parthenocarpic growth of the embryo aids in the appearance of a very small number of seeds in the fruits. Along with this a sharp deformation of the fruits takes place (Fig. 2a) with the formation of a great number of offshoots and swellings, differentiated from the basic mass of the ripe fruit by their green color. Especially strong deformation is observed for fruits of the first flowers; the following flowers are differentiated by less deformation of the fruits and the uppermost flowers have fruit that are not differentiated in form from the fruits of the control plants.

Deformation of fruit is not observed for tomatoes treated with 0.001% and 0.0001% gibberellin (Fig. 2b).

Insofar as we know, this is the first note on the effect of strong deformation of the fruit when tomatoes are treated with gibberellin.

We express out thanks to Professor M. Kh. Chailakhyan for sending us the gibberellin used in the experiments and for his methodological notes on the treatment of test plants with gibberellin.

APPARATUS AND TECHNIQUES FOR STUDYING PLANT ROOTS

N. Z. Stankov

All-Union Institute for Soil and Fertilizer Reaserch (VIUAA), Moscow
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The importance of studying the activity of plant roots is uncontested. Libikh has written that "knowledge of plant root systems is fundamental in agriculture," although the study of them until recently has been conducted in an erractic, unilateral way with study predominantly devoted to the shoot. Rotmister expressed it by saying that the plant root is " the stepchild in science," and this is indeed true. Methodological difficulties are the reason for this situation.

Present methods for the study of root systems are somewhat inexact, tedious and difficult, which deters many investigators interested in root systems.

One of the principal functions of the root system is its interaction with the soil and microflora, which facilitates the processes of nutrition and water supply. However, the existing methods for studying root systems are unsatisfactory for gaining insight into this function of roots. Roots in soil can be studied only on a weight basis or morphologically described on the wall of a trench in the soil. It is extremely important for us to know the distribution of the roots which absorb salts and water from the soil. We recognize that this function is carried out by the fine young roots covered with root hairs. These very roots are partially lost during root measurements by the weight method and even during a careful study their contribution to over-all root weight is so small that the weight method does not represent them reliably. Those roots which weigh much play a slight role in nutrition, as Sabinin [1] has said.

Naturally, we would like to determine not only root weight but also root length. In a determination of root length the fine, physiologically active roots would be as well represented as the old, coarse roots. The mass of fine filamentous roots, frequently a web interlacing the soil, in the weight method have such a minute weight that it is lost in the greater weight of the coarse old roots. In an assessment of root length the fine roots contribute greatly to characterizing the root system.

Existing methods for measurement of root length are imperfect and inexact. Maksimov [2], in his very widely distributed textbook on plant physiology, and also other workers give figures in the length of roots of a single plant which are of astronomic magnitude: total length of roots of about 600 km and length of root hairs 10,000 km. The length of the roots increases 5 km and the root hairs 80 km per day. These data were extracted from the work of Dittmer [3]. They were obtained as follows: The investigator raised winter rye in boxes 30 × 30 and 56 cm deep filled with soil. Only one plant was left in each box. In a subsequent investigation this same researcher [4] also experimented in a sown field of winter rye with core samples 7.6 cm in diameter to a depth of 15 cm. The calculated root lengths obtained in this experiment were completely different. In the first (box) experiment the total length of the roots of a single plant was 622 km; in the second (field) experiment only 63 m. Such a differenct in the calculated length of the roots in the same type of plant (winter rye) can be explained. In the first case (box-grown plants) the length of the roots of a single plant growing in a quite large box was determined while in the second case (field grown plants) the winter rye developed in a plant association.

It is impossible to call Dittmer's method for determining root length a satisfactory one. The root system, washed free of soil, was put in 5% formalin, and then the root system was measured. The number of main seminal roots, secondary roots branching from the main roots, and the number of third and fourth order roots was determined. While the number of main and secondary roots was determined directly, the number of third and fourth order roots and their length was calculated from the average length of roots in a sample of the roots, examined under the microscope. Such a determination, based on an average value, conceals the inherent measurement uncertainty and to what extent the third and fourth order roots contribute to the total length of the root system.

Root hairs were determined as follows: The number of root hairs on 1-mm pieces of root taken from various zones of the roots of a given order were counted under a microscope; the average count obtained was converted to a whole root basis. In this way the number and length of root hairs were determined on roots of the first, second, third, and fourth orders. Dittmer assumet that root hairs were distributed throughout the entire root system and therefore clearly obtained exaggerated values. He calculated the length of root hairs in a winter rye plant growing

Average Length of Roots of a Single Plant in the Plow Layer (length of root without root hairs)

Crop	Av. no. plants/m²	Soil volume/plant, dm³	Average root/plant, m	Average surface, cm²	Average root volume, cm³	Cumulative root volume		Year
						cm³	%	
winter wheat	400	0.5	26.0	204	1.3	32.0	6	1954*
			20.5	165	1.0	25.2	5	1954
spring wheat	300	0.7	79.5	628	4.0	97.8	20	1954
			61.1	479	3.0	15.2	15	1954
			17.6	141	0.9	21.7	4	1957
winter rye	400	0.5	26.0	204	1.3	32.0	6	1956
flax	400	0.5	45.0	353	2.3	55.4	11	1956

* 1954 had a severe spring-summer drought, which affected particularly the spring wheat; it was 15-20 cm tall at anthesis but the length of roots was much greater.

alone (in a box) to be 10,625 km, while that of winter rye grown in a field community was calculated to be much less, only 16.7 km.

A similar method for assessing length of roots has been worked out by Pavylechenko [5]. He transplanted grasses and their associated weeds by the following method. The plants were raised separately without competition from other plants on plots of 0.9 m². The plants were transplanted 15 cm apart in rows, and weeds were transplanted between the rows of grasses.

During the vegetative period soil samples were taken in order to determine the length of roots. On day five a block of soil 229 cm³ was removed, on day 22 one of 46 dm³, on day 40 one of 70 dm³, and on day 80 (ripening period) a 1081 dm³ block of soil. The root system was

Auger for taking soil samples.

washed free of soil, and the length of roots per plant was determined. During the ripening period the following data were obtained.

	1	2
winter rye	77 km	960 m
winter wheat	70 km	853 m
wild oats	87 km	961 m

In the mixed grass and weed plantings the following data on length of roots were also obtained 40 days after interplanting: barley with wild oats, barley 192 m and wild oats 58 m of roots; winter wheat (155 m) with wild oats (58 m); wheat (89 m) with mustard (162 m).

The results of root length determinations by Pavylechenko agree completely with those of Dittmer. In plants growing alone without competition from other plants the length of roots was very large, tens of kilometers per plant. In those plants growing in a plant community the length of roots was much smaller, only hundreds of meters.

Neither workers say anything in their work about the great tediousness and difficulty of it. This kind of method for determining the length of roots can be considered acceptable only when all steps of the operation are executed with the greatest care, and it is necessary to admit that its use will not be widespread.

Savvinov [6] proposed the best method for determining length of roots. His procedure for obtaining roots was adapted to situations where the soil itself was being examined. Samples were taken on the face of a pit in the soil. We modified his procedure for root sampling applied to field conditions but retained the fundamental principles of the method. We took soil samples with a small auger with a shank 50 cm long screwed onto a cylinder with a volume of 50 cm³ (figure). The cylinder contained a horizontal slit which permitted the soil sample to be cut into two equal pieces of 25 cm³ with a knife. In the upper layers of the soil we took a 25 cm³ sample and in the lower layers 50 cm³. This sampler permits samples to be taken in experimental plots without damaging the crop.

Soil samples taken with the tube are put into cans (it is not possible to put them in bags or sacks since the roots would be damaged during the drying process). All subsequent work of root separation is carried out in the laboratory. The roots are cautiously washed out onto a 0.25-mm screen and transferred into clean water. A band of glue is spread on a sheet of paper and allowed to dry. The rootlets are picked up with forceps and placed in a straight line on the band of glue on the paper. All branches on the rootlets are cut off and placed end to end in a straight line. After the entire sample is worked over the rootlets are glued on the paper; their cumulative length is measured and converted to a dm^3-of-soil basis. The diameter of the roots on these sheets may also be measured under a microscope or binocular viewer using reflected light and an ocular micrometer. Knowing the length and diameter of the roots, it is possible to calculate the volume and surface of the roots (using the formula for a cylinder).

In our method sampling the roots is no great task. The bulk of the effort is laboratory work, which, however, can be done anytime. About 10 meters of roots can be glued on in one day.

By this method the length of the roots of grain crops and grasses can be determined, but it can also be used on a wide variety of crops. From the work we have done with this method we have come to the following conclusions: Three replicates are sufficient in uniform stands of the crop but four or five are better. Samples taken can be stored all winter since no particular changes occur in the roots during storage. Where does one take the samples in the field, in the row, alongside the row, or between rows? Our investigation of this shows that the lengths of the roots in all three cases are similar. Therefore, we judge that it is best to take the samples within the rows.

We attempted to calculate the length of roots per plant. Our results, shown in the table, were obtained in the following way. The number of plants per square meter of the experimental field was evaluated (400 plants of wheat and winter rye, 300 of spring wheat, and 400 of flax per square meter). The volume of soil in the plow layer, in one square meter to a depth of 20 cm, is 200 dm^3. By dividing this soil volume by the average number of plants per square meter we obtained the volume of soil occupied by one plant. This volume is 0.5 dm^3 for winter wheat and flax and 0.7 dm^3 for spring wheat. Using the values for root length, we calculated the average length of roots per dm^3 of soil 0-20 cm deep for a given crop, and from these average values we calculated the length of roots in the volume of soil occupied by one plant. These values express the length of roots per plant in the soil plow layer. From this value we calculated the root surface and volume, using the formula for a cylinder.

The root hair method of Savvinov [6] does not give the length of the roots; therefore we approached this in another manner. Having obtained an average length of root hairs of, say, 0.5 mm we assume that root hairs cover the entire root surface at some time (youth). All soil within 0.5 mm of the root surface was permeated by root hairs. Because of the enormous surface of the root hairs, the entire volume of soil penetrated by them would be "touched" and "tapped." Knowing the length and surface of the roots, we can calculate the volume of soil lying within 0.5 mm of the root surface. We call this soil volume the layer of soil "attached" to the roots, expressing it in cm^3 and percentage of the soil volume occupied by one plant. The dimensions of this near-root layer we characterize as the zone of root hair activity. According to contemporary ideas, the process of nutrient flow into the root cells consists in contact exchange between the physiologically active root surface and the surface of the soil particles. Therefore, from the dimensions of the near-root soil layer we can judge the conditions for uptake of nutrient elements and water by the root cell and also the extent of root hair activity.

The data we have obtained on length of roots in a single plant ought to be considered preliminary inasmuch as they have been calculated for a relatively small number of plants. They can be used only for testing the correctness of the methodological approach. The values for surface area of roots, root volume, and the volume of the soil "attached" to the root have been obtained before. They much extend our ideas concerning the activity of roots in soil.

In conclusion it can be said that Savvinov's method for measuring the length of roots with our additions of a soil sampling method can be fully recognized as suitable for use in scientific research.

SUMMARY

A method for determining the length of plant roots under field conditions is described. The soil auger with a rod of suitable length and a 50 cm^3 cup is used. Samples from small experimental plots can be taken with such an auger without disturbing the crop.

The soil samples are dried in air in the laboratory, and the roots extracted from them. For this purpose a small part of the soil is soaked in water, and the roots are removed with tweezers and a magnifying glass and glued in a line on paper. In this way the total length per 50 cm^3 can be determined. The density of the plants in the field is then determined, and the soil volume per plant is computed. Knowing the root length in a volume of soil at a given depth one can compute the root length per plant. Tests of this method have shown that it offers some new opportunities.

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A SIMPLIFIED LABORATORY APPARATUS FOR RAPID FREEZE-DRYING OF BIOLOGICAL MATERIALS

B. B. Vartapetyan

K. A. Timiryazev Institute of Plant Physiology. Academy of Sciences of the USSR, Moscow

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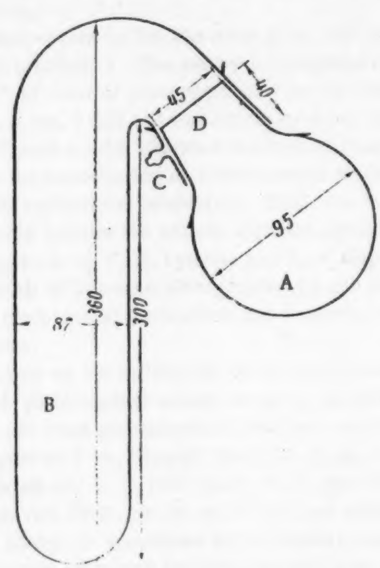
Drying of biological materials by rapidly freezing them and drying them in the frozen state is preferable to other methods of fixing material since the freeze drying method preserves them biologically and chemically unaltered [1]. The wide use of this technique in laboratory practice requires a vessel of sufficiently simple construction and easy to use.

We have already presented an apparatus to a certain extent satisfying these requirements [2]. This apparatus has been quite widely used in various laboratories, which prompted us to improve it further.

The apparatus described in this paper is simpler and improved, which makes its use more convenient on the one hand and improves the quality of the freeze-drying on the other hand, since changes in construction have almost halved the distance between the surface that is drying and the condenser. This is especially important since the pathway for the molecules of solvent under vacuum is reduced compared to drying carried out in an apparatus when the atmosphere is not evacuated (see below). This permits an increase in the amount of material dried per unit time compared with the amount previously dried in an apparatus of the same dimensions [2].



Sketch of apparatus for rapid freeze-drying of biological material (dimensions in mm).

The apparatus (see figure) is made completely of glass put together in two parts: a flask (A) and a condenser (B). The condenser has an exit tube (C) for connection to an ordinary electrical oil vacuum pump. There is an opening of 5 mm in diameter in the flask (A) located where the letter (D) is shown in the sketch.

Drying is done as follows: The material to be dried is placed in the flask (A), which is then rotated to match the hole (D) in the neck of the flask with the vacuum tube (C). The contents of the flask (A) are swiftly frozen by submerging the flask in a mixture of acetone or ethyl alcohol and solid carbon dioxide (dry ice). If the material to be frozen is a solution, then for rapid drying it is desirable to spread the solution over the wall of the flask during the freezing process. Then the apparatus is evacuated with the pump (about 10^{-1} mm mercury), the flask is rotated to close the opening (D) and cut off the atmosphere from access to the flask, and the condenser (B) is submerged in a Dewar flask containing liquid nitrogen or a mixture of acetone or ethyl alcohol and dry ice. The vacuum tube can then be disconnected at (C).

While the air is being evacuated from the apparatus (2-3 min), a freeze-out trap is placed between the pump and the apparatus to prevent the solution from being sucked over into the pump and to rapidly create vacuum conditions in the apparatus.

If the drying proceeds normally, the flask (A) need not be opened until drying is concluded since the condenser (B) is submerged in the freezing bath, and the large temperature gradient between them evaporates the ice from flask (A) to condense in (B). This causes a pronounced cooling of the contents of flask (A) despite the fact that the ambient temperature about flask (A) is room temperature. Drying is complete when the outside wall of the flask (A) is free of ice condensed on it from the atmosphere during the drying process.

When drying is complete, air is readmitted to the flask and the dry preparation is removed. Air should be

let in cautiously to prevent the dry preparation from being swept into the condenser (B).

The apparatus can be made with different dimensions depending on the amount of material being dried. In an apparatus having the dimensions shown in the sketch, one can dry a volume up to 100-125 ml of solution. In such an apparatus material can be freed of not only water but also freed of organic solvents (alcohol, acetone, ether). In this event the solution is frozen in liquid nitrogen before the air is evacuated from the apparatus. Because of the low freezing points of organic solvents drying occurs from very cold solutions rather than from the solid state.

As already mentioned, evacuation of the air from such an apparatus is not continuous, but for only 2-3 min. This is an advantage of the apparatus since it permits drying to be done outside of working hours (e.g., night). Since the pump does not need to be used continuously, this feature of the apparatus permits the use of several apparatuses and a single vacuum pump to dry a large amount of material.

The apparatus can be used to concentrate protein or any solution. It can be of use in chemical laboratories for drying reagents unstable at high temperatures and also in work with heavy water.

SUMMARY

The description and application of a laboratory apparatus for drying biological materials by rapid freeze-drying are given.

The apparatus is made completely of glass and consists of a flask (A) for the material to be dried and a condenser (B) which is immersed in a cooling mixture after a vacuum (about 10^{-1} mm Hg) is established in the apparatus. It is not necessary to evacuate the apparatus continuously. With the dimensions employed it is possible to dry up to 100–125 ml of solution.

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REVIEW

Bulletin of Plant Physiology,* Nos. 1-5,† 1958, 1959

National Editor Academician P. A. Vlasyuk
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Reviewed by G. V. Porutskii

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The Ukranian Institute of Plant Physiology has undertaken the publication of a new journal, "Bulletin of Plant Physiology." Its publication is quite appropriate since the rapid development of plant physiology in the Ukraine has resulted in the appearance of a large number of papers.

The First Ukranian Conference on Plant Physiology, convened in Kiev in 1958, registered 65 Ukranian research workers from higher schools who are connected with phases of plant physiology.

In addition to general experimental work the "Bulletin" presents Ukranian physiologists with new problems whose solutions are important to the further development of agriculture.

The close relation between science and practice was emphasized in the principal resolutions of the Twenty-First Congress of the Communist Party of the Soviet Union.

To what extent do the Ukranian plant physiologists follow this principle? The answer is contained in the editorial," 40 years of plant physiology in the Ukraine" (Bull. No. 5, pp. 3-12) and the article by P. A. Vlasyuk, "Results of work by the Ukranian Institute of Plant Physiology and the introduction of achievements of the Institute into agricultural production" (Bull. No. 2, pp. 3-15). In these articles the authors show the significance of investigations by T. D. Lysenko and A. A. Sapegin in the growth of Ukranian plant physiology and have analyzed phsiological works which are a continuation of these studies.

New data on the utilization of microfertilizers as a result of physiological studies on trace elements, vitamins and other physiological substances are presented in the papers by P. A. Vlasyuk (Bull. No. 1, pp. 6-11), P. A. Vlasyuk and V. S. Dol' (Bull. No. 5, pp. 45-50) K. P. Butkevich (Bull. No. 3, pp. 67-72) and others.

The change to granulated superphosphate containing manganese improved fertilizer spreading on mixing with nitrogen and potassium fertilizers; speeded growth and development; and increased photosynthesis, yield, and crop quality.

New data on the physiology of plant cold tolerance and frost resistance are presented by M. A. Berkley (Bull. No. 2. pp. 66-73), R. F. Protsko (Bull. No. 2, pp. 61-66), N. A. Fedorova, M. A. Burileva, and N. V. Kostian (Bull. No. 1, pp. 24-34) and S. K. Ovechkina (Bull. No. 5, pp. 12-25).

Varied temperature (+15; −3°C) for 10 days is recommended for increasing the cold tolerance of buckwheat, and seed vernalization and growing the seedlings on shortened days for increasing the cold tolerance of peaches.

As a rule, the high frost resistance of varieties of winter wheat is because of the limited breakdown of nucleoproteins under frost conditions, a lower content of free water in the plasma and a high content of soluble sugars and proteins.

The rate of P^{32} absorption by roots of winter wheat because of damage during prefreezing and the nature of selective distribution of P^{32} by the organs seem different at different times under field conditions.

The phenomena of organogenesis and embryogenesis of higher plants from the phasic angle and in relation to ontogenetical changes of a physiological and ecological nature, discussed in the work of F. L. Kalinin (Bull. No. 5, pp. 32-37) and E. D. Buslova (Bull. No. 4, pp. 50-58), facilitate the drawing together of the morphology and physiology of plant development. The variation of the characteristics and capabilities of plants, their cold resistance, and the duration of the vegetative period depends upon the phase during which external factors (temperature, etc.) affect the organism.

Investigation of genetic problems is also not without significance. In the development of plant phys-

* By this note the editorial board of "Plant Physiology" calls attention to its wide circle of readers and the emergence of the "Bulletin of Plant Physiology"; heralds its publication as an important event; and congratulates the Ukrainian plant physiologists.

†Number 5 of the "Bulletin of Plant Physiology" contains a number of scientific papers of the Institute.

iology, for example, the paper of P. M. Shul'gin (Bull. No. 3, pp. 21-30) attacks the problem of the physiological interaction between root stock and scion. The papers of P. A. Vlasyuk and L. D. Lendensko (Bull. No. 4, pp. 3-10) and G. Kh. Molotovskii (Bull. No. 2, pp. 15-21) on polarity and plant quality relate to this.

In plant physiology the Soviet Ukraine is developing along the lines of thought of E. F. Votchal, V. N. Lyubimenko, and N. G. Kholodnii.

The research of E. F. Votchal on sap movement in plants is reflected in the papers of L. A. Filip'eva (Bull. No. 3, pp. 60-67; No. 5, pp. 25-32) in which is given the physiological basis for one of the most important physical soil-water constants in irrigated agriculture. The quality of harvested plants is considered as a function of these criteria.

The papers of K. D. Kolomiets (Bull. No. 4, pp. 77-82), P. A. Vlasyuk (Bull. No. 3, p. 370), and Z. M. Klimovitskii and K. L. Vizir (Bull. No. 5, pp. 63-69), which are related to the investigations of V. N. Lyubimenko, concern the effect of light on physiological processes of plants and the study of chlorophyll and other plant pigments.

K. D. Kolomiets has established the relationship between the quality of tea leaves and their pigment content and has constructed special apparatus for partitioning of pigments. P. A. Vlasyuk and Z. M. Klimovitskii have studied the ability of proteinaceous compounds to generate metabolites having exceptionally great activity in relation to photosynthesis. It has been shown that chlorophyll synthesis is accelerated by manganese, copper, and zinc, while copper and manganese promote increased stability of the chlorophyll protein bonding.

The papers of S. N. Dolgoi (Bull. No. 2, pp 53-61; No. 4, pp. 27-31), which are a continuation of the work of N. G. Kholodnii on plant electrophysiology, establish a relation between the rate of uptake and the vigor of initial growth and the internal electrophysical processes while proposing an electrophysiological method for determining the viability of hybrid seeds.

The paper of Kh. N. Pochinka concerns questions of techniques: the determination of glucose, fructose, and sucrose in plants on a single sample by the iodometric endpoint of separation of cuprous oxide (Bull. No. 2, pp. 27-34); a chloramine method for determination of nitrogen in plants (Bull. No. 3, pp. 42-45); determination of sucrase and amylase activity (Bull. No. 4, pp. 42-45); determination of starch in leaves and other plant organs by volumetric and colorometric methods (Bull. No. 5, pp. 59-63), etc.

Thus, it is true that the material in the "Bulletin of Plant Physiology" reflects an appeciable movement of plant physiology toward solution of active problems in agriculture. The role of N. G. Kholodnii (Bull. No. 2, pp. 73-81), E. F. Votchal (Bull. No. 4, pp. 82-86), V. N. Lyubimenko (Bull. No. 5, pp. 84-87), and other Soviet scientists in the development of experimental plant physiology in the Ukraine has been shown with great conviction.

Markedly fewer papers were concerned with the question of the physiology of plant hormones, features of the phasic changes in plant development in relation to climatic conditions and cultural conditions of each crop and the physiology of vigor and heterosis. Elucidation of these questions is a future task for the staff of the Institute.

The publication of a journal on plant physiology in the Ukraine is an important beginning, and it doubtless will promote progress in experimental physiology and agriculture.

EVENTS

CONFERENCE ON MEASUREMENT OF VISIBLE RADIATION IN PLANT PHYSIOLOGY, AGROMETEOROLOGY, AND ECOLOGY (APRIL 20-21, 1960)

A. A. Nichiporovich

Translated from Fiziologiya Rastenii, Vol. 7, No. 6, pp. 744-747, November-December, 1960

For plants, solar energy is one of the most important environmental factors. Recently there has been a widespread development of research using plants raised under artificial light sources.

Photobiology is becoming one of the important and rapidly developing branches of biology. Almost every year we are discovering new plant photobiologic reactions and new acceptors of light energy which govern the many-sided, complex, and delicate relations between plant vitality and solar and artificial sources of radiation.

In contemporary work many highly important theoretical and practical problems of plant physiology and high crop productivity cannot be solved without knowledge of the amount of radiation received by different parts of the spectrum and exact knowledge of their physiological activity.

Because of this it is necessary to put into the hands of investigators and practical workers convenient and dependable methods for measuring radiation in various parts of the spectrum in strict accordance with their physiological role.

However, our knowledge in this area can not be considered satisfactory: Radiation in certain spectral regions has a specific physiological significance and ought to be considered in certain cases; the required instruments and techniques of measurement have not been standardized, and agreement has not been reached on common units of measurement and expression.

In order to correct these deficiencies and to provide greater unanimity regarding techniques of measurement and requirements for future instrument design, a conference has been held at the Institute of Plant Physiology of the Academy of Sciences, SSSR.

The workers of a number of institutes and other establishments participated in the conference.

Yu. D. Yanishevskii (Central Geophysical Observatory), V. P. Rvachev (Baskir Agricultural Institute), B. I. Gulyaev (Institute of Plant Physiology, Academy of Sciences, USSR). M. V. Sokolov (Institute of Biophysics, Academy of Sciences, USSR), A. A. Nichiporovich, A. F. Kleshnin and L. N. Bell (Institute of Plant Physiology, Academy of Sciences, USSR) were appointed to a standing commitee to actively work on the problems of measuring visible radiation in plant physiology. V. M. Leman (Timiryazev Agricultural Academy), V. L. Voznesenskii (Botanical Institute, Academy of Sciences, USSR), I. A. Pyrina (Institute of Biological Water Supply, Academy of Sciences, USSR), S. B. Gurtman (Factory "Vibrator"), I. I. Sventitskii (Institute of Rural Electrification VASKhNIL), S. V. Tageeva, A. B. Brandt, M. B. Zimin (Institute of Biophysics, Academy of Sciences, USSR), S. N. Chmora and N. N. Protasova (Institute of Plant Physiology, Academy of Sciences, USSR) were also selected as members of the committee.

The resolutions of the committee published below should assist in systematizing work in study and control of the interaction between plants and light.

MEASUREMENT OF VISIBLE RADIATION IN PLANT PHYSIOLOGY AND ECOLOGY, AGROMETEOROLOGY AND PLANT PRODUCTION

(Resolutions of the working committee on the problems of measuring light energy in plant physiology of the K. A. Timiryazev Institute of Plant Physiology, Academy of Sciences of the USSR)

1. Visible radiation absorbed by plant leaves is one of the most important factors of the environment which determine the trend of photosynthesis, the conditions of water supply, mineral nutrition, temperature and in the last analysis plant yield. The effect of visible radiation on plants is determined by the spectral composition of the radiation, the exposure time and the distribution of light in time and space. Therefore, determination of the spectral composition of radiation and changes in the duration and amount of radiation is an important condition for exact physiological investigations in agrobiological experiments, agronomic practice, and ecological observation.

2. Absorption by the normal green leaf is described by a single bimodal curve: of the order of 90% absorption in the near-ultraviolet and blue-violet region (300-480 mμ) and red wavelengths (650-680 mμ) and a minimum of about 70% absorption in the green region (520-560 mμ). Absorption falls abruptly from 90 to 10% between 680 and 740 mμ, remains at 10% in the near infrared wavelengths (740-1200 mμ), and gradually rises again to 90% near 1500 mμ.

Apparently under conditions of natural insolation absorption in the ultraviolet region (300-710 mμ) commonly reaches 80%, and about 10% in the infrared region (740-3000 mμ).

3. The most important physiological processes connected with absorption of visible radiation, photosynthesis, pigment synthesis, photoperiodism, and movement of plastids and plant organs (phototaxis, phototropism) are governed chiefly by radiation in the region of approximately 380-710 mμ. Wavelengths near the infrared region (720-800 mμ) are also active for a number of growth processes and seed germination; ultraviolet and radiation of wavelength below 300 mμ (lethal radiation) cause plant death. The physiological importance of ultraviolet radiation in the region of 300-380 mμ and the region of infrared and longer wavelengths (720-1200 mμ) has still not been studied sufficiently. Infrared and long-wavelength radiation of more than 1200 mμ can affect plant development by altering the temperature regime of the plants and of the surrounding environment (soil, air, and thus plant organs).

4. Natural insolation (solar) and artificial sources (incandescent, fluorescent, and gas lamps) are usually concentrated between 300-3000 mμ, but these light sources differ in their spectral distribution of energy.

5. The objectives and problems in measuring light energy during work in plant physiology and ecology, agrometeorology, and plant production are diverse, and they determine the different requirements for instrument construction, the technical features of the measurement apparatus, and the differing ways in which they are used.

Instruments intended for measurement of light energy can be classified on the basis of their spectral sensitivity, the shape of the receiver (e. g., plate or sphere), and the time characteristic of the recorder (instantaneous or integral).

6. The committee considers that a nonselectively sensitive apparatus should be used before obtaining sufficiently reliable data on the spectral activity of physiological processes, primarily photosynthesis. Both the minimum of photosynthetically active radiation (380-710 mμ) and the physiologically most active region should be measured. In a number of cases, particularly during study of the energy balance of a crop, it is also necessary to determine the intensity of radiation from 710-3000 mμ.

Bearing in mind that the different areas of the photosynthetically active spectrum have unequal physiological effects we recommend the measurement of radiation over three separate spectral intervals: 1) 380-535 mμ; 2) 535-610 mμ; and 3) 610-710 mμ. These spectral regions not only have a recognized degree of physiological specificity, but they also correspond to regions of solar energy considered by stations of the weather services through international agreement. Therefore, by dividing photosynthetically active radiation in this way it is possible to use data obtained from meteorological stations.

7. To quantitatively characterize visual radiation in physiology, plant ecology, and agrometeorology primarily requires measurement of the rate of illumination (visible energy received per minute per unit area of illuminated surface) and the amount of radiation or dose (i.e., the cumulative amount of illumination received during the illumination period). The committee considers that before sufficiently complete data are obtained on the active spectrum of the most important physiological processes it would be premature to introduce new units to characterize radiation on the basis of its effect on plants. Therefore, use of the generally employed units is recommended: for illumination rate cal/cm^2/min, cal/dm^2/hr, ergs/cm^2/sec, w/m^2, and correspondingly for cumulative amount of radiation, cal/cm^2, ergs/cm^2, w-hr/m^2, etc.

8. Usually the following are determined in practice:

a) Horizontal illumination of an openly exposed surface (macroclimate) or the illumination of separate communities (macroclimate) and finally, illumination of individual plants or plant parts;

b) Cumulative amount of light received by individual plants, crops, or coenoses during some time interval (most often, amount per day);

c) Energy balance of the crop, with the purpose of completely characterizing the radiation and energy regimen of the crop in regard to the amount of light energy received by the crop, reflected from it, absorbed by the plants themselves, and absorbed and reflected by the soil. Such measurements are of particular significance in arriving at a coefficient of efficiency of light use in photosynthesis, dry matter gain, transpiration, determination of the required irrigation regimen, for devising a system for obtaining a stand with optimal density, etc.

9. The measurement apparatus selected depends upon the nature of the problem at hand. For example, investigation of the energy balance of a planted area requires integrating apparatus which separately measures the photosynthetically active and infrared radiation (up to 3000 m μ). Neither can the receiving surface always be the same but must be determined by the nature of the problem at hand. The spherical shaped light energy receiver should be used primarily for the study of the radiation regimen of individual plants (e.g., in the greenhouse) and in the study of the energy balance of crops and illumination within a mass of plants.

However, further study of methods is required in order to finally resolve the problem of what shape the apparatus should have.

10. At present, industry does not make apparatus completely suitable for making the measurements indicated above.

The Yanishevskii pyranometer and the so-called "luxmeter" are among a number of apparatuses produced for the practical measurement of visible radiation.

Preferable among these two instruments is the Yanishevskii pyranometer, which is essentially nonselectively sensitive in the visible region of the spectrum. In conjunction with a suitable light filter (e.g., BS-8, thickness 3 mm, OS-11, 3mm, KS-10, 3 mm, and KS-19, 3 mm) this instrument can be used to separate light according to the regions of the visible spectrum indicated above. Certain deficiencies of this instrument are its selective sensitivity in the near-infrared, its relatively large inertia, and its low sensitivity, which make necessary the use of a sensitive, low-resistance galvanometer.

Recently another instrument, " the luxmeter," has been devised, which really consists of a selenium photoelement with a corrective green filter approximating the spectral sensivity of the apparatus to the median spectral sensitivity of the human eye. Therefore a correction coefficient applied to other luxmeters is not used with this apparatus. One deficiency of a selenium photoelement is the great scatter of the spectral curve of sensitivity of the manufactured instrument and the dependence of its sensitivity upon the angle of the incident radiation. This apparatus can be used for assessing the photosynthetic activity of radiation along a gradient in a given light field. In view of the unstable characteristics of a majority of selenium photoelements being marketed (particularly for field use) a periodic calibration of them is desirable by comparison with thermoelectric instruments like the Yanishevskii pyranometer provided with a suitable light filter.

Someday, special apparatus ought to be designed which is suitable for measurement of photosynthetically active radiation, and which abandons the use of selenium photoelements requiring light filters.

11. Among the instruments which are not mass-produced but which are suitable for measuring photosynthetically active radiation the following can be mentioned.

The photoelectric phytoactinometer of the Institute of Plant Physiology (Bell L. N.) consists of a selenium photoelement covered with a mosaic light filter nearly nonselectively sensitive in the region 400-700 mμ. Consequently, this instrument to a greater degree satisfies the need for nonselective measurement of photosynthetically active radiation than the luxmeter or selenium photoelements without light filters. This instrument has still not attained wide use because of difficulties in manufacturing the mosaic filters.

The photointegrator of the Institute of Plant Physiology, Academy of Sciences, USSR has been used during the last two years for experimental measurement of the radiation energy balance of crops. This instrument is sensitive to photosynthetically active radiation and provides an integral record of it, i. e., the cumulative amount of radiation.

A compensational thermopile, i. e., two identical thermopiles pointed toward one another, is a suitable instrument for separately sensing the individual regions of the visual radiation. By covering one of the thermopiles with a light filter which transmits all of the long wavelengths greater than 380 mμ (e. g., BS-8, 3 mm), and covering the other thermopile with a filter transmitting long wavelengths greater than 710 mμ (e. g., KS-19, 3 mm), a suitable apparatus can be made for measuring illumination in the photosynthetically active region.

By appropriate selection of light filters a suitable arrangement can be made to measure illumination in the infrared or ultraviolet region of the spectrum. However, in devising this apparatus a number of obstacles must be overcome since flat light filters incorrectly assess the obliquely received (scattered) light.

This deficiency can be circumvented by the use of hemispherical light filters (substantially a practical problem) or by use of a diffusion dome, but this appreciably reduces the sensitivity of the apparatus and makes

necessary the use of a very sensitive galvanometer.

12. The committee considers that, despite the existence of these instruments which permit the study of many problems confronting plant physiologists, agrometeorologists, ecologists, and agronomists, at present no instrument is being made which can be considered completely satisfactory.

In regard to this the committee considers that the most important conditions for successful solution of problems concerning the measurement of visible radiation from physiological and other points of view are:

a) The manufacture of new, more exact instruments for nonselective measurement of visible radiation over the spectral intervals 300-380 mμ, 380-710 mμ, 710-3000 mμ and also 380-535 mμ, 535-610 mμ, 610-710 mμ.

b) Acceleration of the work of determining the action spectra of the most important physiological processes going on in plants. Successful consummation of this effort would permit diversification and selection of instruments with curves of spectral sensitivity best matching the physiological peculiarities of the plant and the problem being studied.

13. In order to accomplish the work mentioned above, the committee considers it is necessary to:

a) Request that the Institute of Plant Physiology and the Institute of Biophysics, Academy of Sciences, USSR and the Institute of Plant Physiology of the Ukranian USSR expand research on the action spectrum of photosynthesis and other photobiological processes in plants.

b) Request that the K. A. Timiryazev Institute of Plant Physiology and the Institute of Biophysics of the Academy of Sciences, USSR, the Agrophysics Institute of the VASKhNIL and the Institute of Plant Physiology of the Ukranian SSR speed up development of photointegrators and phytoactionometers which are nonselectively sensitive over 380-710 and 300-3000 mμ.

c) Request that the factory "Vibrator" tool up for production of electrolytic integrators and selenium photoelements of "lux-hour meters" adaptable for work under field conditions and equipped with mosaic light filters for nonselectively registering photosynthetically active radiation.

d) Request that the experimental instrument manufacturing plant of the VASKhNIL speed up production of photointegrators of the FI-1(Institute of Plant Physiology) type.

e) Recommend that the Agrophysics Institute, the factory "Vibrator" and other organizations producing luxmeters state on the instrument plate that these instruments indicate in luxes only when they are used under incandescent lights within the limits stated on the plate.

In all these cases the luxmeters mentioned can be used only for relative measurements of the magnitude of illumination,or else they require use of special conversion coefficients.

f) Request that the A. I. Voeikov Central Geophysical Observatory, the Physics Institute of Leningrad State University and the Aeromethods Laboratory and Institute of Biophysics of the Academy of Sciences, USSR, design field instruments for registering spectral illumination in the regions of 380-710 mμ and 300-3000 mμ.

g) Request that the Central Administration for Weights and Measures of the Soviet Ministry, USSR, direct the Scientific Research Institute of Metrology to organize the examination of spectral characteristics of instruments used to measure visible radiation, particularly photosynthetically active radiation.

h) Request that the Central Hydrometeorological Service and the observatories located in the most important agricultural regions of the country make regular measurements of photosynthetically active radiation and direct appropriate work at agrometeorological stations.

i) Request that the A. I. Voeikov Central Geophysical Observatory work out methods for measuring photosynthetically active radiation and direct appropriate work at agrometeorological stations.

j) Request that the Central Geophysical Observatory work out a standard apparatus for measuring illumination in the regions of 300-380 mμ, 380-535 mμ, 535-610 mμ 610-710 mμ, 710-3000 mμ and for testing the various instruments proposed for measurement of visible radiation in the fields of plant physiology, plant production, etc.

VISIT TO PLANT PHYSIOLOGISTS IN ENGLAND AND SCOTLAND

A. L. Kursanov

K. A. Timiryazev Institute of Plant Physiology, Academy of Sciences of the USSR, Moscow

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The first time the author of this review visited Great Britain was in the fall of 1955, when he had the opportunity to become acquainted with the organization of several scientific institutes and universities of that country, as well as with the studies on plant physiology conducted there. These impressions were reported in "Plant Physiology," 3, 2, 179 (1956).

The author again visited Great Britain in May of this year, this time to lecture on plant physiology to students, instructors, and scientists. Three lectures were prepared for this occasion: 1) Plant Physiology in the Soviet Union; 2) Physiology of the Intact Plant; and 3) The Transport of Organic Substances. These lectures were delivered in London, at the Imperial College and Kings College; in Kent County, at Wye College, and also at Cambridge, Oxford and Edinburgh Universities. As a result, the author had the opportunity of visiting many large universities and colleges of Great Britain in a relatively short period of time (15 days). Apart from delivering lectures the author had the opportunity to examine the laboratories in detail and to learn about the experiments conducted there. The author knew some of these institutions from his first visit; he also had made the acquaintance of a number of scientists previously. Therefore, a comparison of the present activities of these laboratories with those seen 5 years ago was of special interest, since it helped in understanding developing tendencies in certain phases of plant physiology.

Great Britain is a country where plant physiology has long occupied a prominent position. This is shown by its scientific literature. Of course, one is best graphically convinced of it on personal meetings and visits to laboratories.

The visit in 1960 convinced the author that the specific importance of plant physiology among other botanical training studies has been accelerated greatly during the elapsed period. This was manifested primarily in the great progress attained in the study of all phases of plant metabolism, as well as in widening the sphere of physiological effects in adjacent fields of knowledge.

Plant physiology in British universities is a division of the botany department which, in accordance with the old traditions, is headed by taxonomy or morphology professors. The exception was the Imperial College of London, where there was an independent plant physiology department headed by Professor W. James.

In recent years the initiative and the activity of the botanical department is devoted mostly to plant physiology. In some universities, in Edinburgh for example, the herbarium and the descriptive section of botany are centered in the botanical garden, while the buildings of the botanical department are devoted to physiological laboratories. At the same time another special building is being constructed for studies in plant physiology. In addition, studies on plant physiology are widened in scope in the agricultural departments of universities and in agricultural experimental stations. Particularly at Oxford, the agricultural department, headed by Professor B. Blackman, in recent years stresses the physiological theme on an even wider plane than the botanical department of that university. Plant physiology is also very seriously applied at the Rothamsted experimental station, in the agronomy department of London university, at Ashford and at other places.

Characteristic achievements are notable in the study of plant physiology itself. Even greater attention is being paid to the problems of metabolism, i. e., to the problems of internal organization of physiological processes. Quite substantial results were achieved in recent studies particularly in this direction. In all plant physiology laboratories the most serious investigations of a biochemical charac ter are being conducted in which not only are biochemical methods used, but the very foundations of biochemistry are tested. Thus an impression is created that in the process of scientific development, the boundary line between plant physiology and biochemistry becomes ever narrower. Contemporary plant physiology is converted into a science which

studies the active life of plants on a foundation of their biochemical organization. Perhaps this is the most characteristic feature in the development of contemporary plant physiology.

In turning to the review of individual studies, we of necessity can have only a cursory glance at a few. Nonetheless, we shall endeavor to review the type of studies characteristic of individual scientific institutions or those which are similar to the studies conducted in the Soviet Union.

The Rothamsted experimental station is devoted chiefly to applied plant physiology. Here Dr. Watson and his collaborators, as formerly, are occupied with a study of the photosynthetic apparatus in cultivated plants as related to field productivity. Their main conclusion is that plant productivity is determined primarily by the dimensions of the assimilative surface, and not by the intensity of the photosynthesis itself. Therefore, Watson [1] considers very important for total field productivity the earliest possible development of the leafy apparatus, which would enable the cultivated field fully to utilize the solar radiation falling on it in the first weeks of the growth period.

These conclusions and the results of measurements are very similar to the data obtained by A. Nichiporovich [2] in the Soviet Union.

In developing these studies further, the Rothamsted physiologists undertook a comparative study of the leafy growth and photosynthesis intensity in cultivated plants and the wild forms closest to them. It was found that the wild forms, for instance Beta vulgaris subsp. maritima [3] or Avena fatua [4], have approximately the same photosynthetic intensity and the same relationship to nitrogen nutrition as the cultivated counterparts.

Thus, it must be concluded that the greater productivity of cultivated plants depends on a better development of their leafy apparatus and a more favorable exposure of the leaves.

However, besides the leaves, the other green parts of the plant also participate in general field productivity. Their share is especially prominent in grains. According to G. Thorn's data [5] the photosynthetic intensity of barley leafy casings is approximately equal to the photosynthetic intensity of leaf blades, but due to the fact that the casing encloses the vigorously respiring stem, its productivity (per dm^2) is equal only to $^1/_2$ of the leaf productivity.

Watson, Thorn and French [6] also found in barley that nearly 26% of the organic substance of the seeds is produced at the expense of photosynthesis within the spike itself; nearly 59% comes from leaf blades and up to 15% from other parts of the plant. It is of interest to compare these results with data obtained in the author's laboratory [6a], according to which the bolls of the cotton plant play an essential part in supplying the developing grains and fibers with assimilants.

Of interest are Owen and Watson's observations [7], which showed that sugar beets that withstood prolonged drought sharply increased their leaf growth and accumulation of organic substance even with slight moisture precipitation, which cannot compare with the water reserve created in the soil upon artificial irrigation. It is possible that this phenomenon has the same biological foundation as the response of tea plants to slight moistening by sprinkling in a period of drought (see N. Petinov and G. Lebedev [8]).

Just as in the Soviet Union, serious attention is paid in England to the physiological basis of nonroot feeding. Particularly in Rothamsted, Thorn [9] has shown with the aid of P^{32} that sodium phosphate applied to turnip leaves and kidney bean leaves is well absorbed and is quickly transmitted to other organs. However, with simultaneous increase in phosphate feeding through roots, the phosphate absorbed by leaves remains in situ. These results illustrate well the interrelation between root and nonroot feeding of plants, which was also found in the Soviet Union by A. Pavlov [10], V. Ivanov [11] and others.

Finally it should also be noted that the activity of gibberellic acid on plant growth is being studied at the Rothamsted experimental station. To the author's knowledge, no fixed results of practical value have so far been obtained. According to observations of E. Humphries [12] spraying of young potato plants with gibbererellic acid considerably increases growth of leaves and tuber formation at first. However, later these differences are smoothed over, i.e., in subsequent periods the growth of experimental plants slows down by comparison with the control even on repeated applications of the stimulant. An impression is created that gibberellin applied in the initial stages of growth causes only a speedier manifestation of the plant's "growth potential," but does not exert (at least not in potatoes) any prolonged stimulation of growth processes. Similar data were obtained by Humphries [13] also on growth stimulation by gibberellic acid of the primary leaves of Phaseolus vulgaris. These data could possibly be of practical value, for instance, in accelerating the development of the leafy apparatus in young plants, which is important to better utilization of solar radiation falling on the field. However, as also pointed out by Humphries, leaves whose growth is activitated by gibberellin are frequently poor in chlorophyll. Therefore, this author's attempts to combine the action of gibberellin with kinetin deserves attention, since K. Mothes, E. Engelbrecht and O. Kulaeva [14] found that kinetin aids in the accumulation and preservation of chlorophyll in leaf blades.

Eighty miles southeast of London, near the Channel coast, is the site of Wye College, which is the agricultural department (faculty) of London university. Kent county, where the college is located, is the farming

center of England, predominantly horticultural and with numerous fields devoted to hop cultivation. Wye College, under D. Skilbeck, has 850 acres of land, a large farm, hothouses and laboratories. Here, as everywhere in England, the process of study is closely linked with experimental research.

Among the scientific studies on plant physiology and biochemistry special mention should be given to studies by Professor R. Wain and his collaborators on the nature of selective action by herbicides. In particular, it was shown that in the homologous series of 2-methyl-4-chlorophenoxy derivatives only the acetate derivative is toxic to all plants. The higher homologues with an odd number of atoms in the side chain are completely inactive on plants. As fas as the derivatives of butyric, caproic and other acids with an even number of carbons are concerned, they are just as inactive by themselves as are the compounds with an odd number of atoms. However, as was shown experimentally [15], many plants contain an enzyme capable of β-oxidation of side chains with an even number of carbons. This brings on in the final stage a conversion of such derivatives into a toxic acetyl derivative (see bottom of page).

Particularly thistle, mustard, nettle and some other plants belong to these. At the same time clover, peas, and tomatoes do not contain any active β-oxidase and therefore are sensitive only to direct action of acetyl derivatives [16,17].

Thus, a new logical principle is uncovered by these investigations in the choice and synthesis of selective herbicides. Most probably in the future such a choice can be based not only on the mechanism of β-oxidase, but also on differences in the plant's enzymatic apparatus.

At present Professor Wain is investigating the nature of the intermediate products of herbicide conversion in plants. He also is closely studying the conversion of selectively active herbicides in the soil as affected by microorganisms; this is very important, since in some cases it may cause a poisoning of the crops themselves. All these studies are financially supported by the British Royal Society for agricultural studies.

An example of these investigations indicates how fruitful a theory of physiologically active substances can be found to be. Investigations carried on in the Soviet Union by Yu. Rakitin and his collaborators [18,19], as well as N. Mel'nikov [20] and some others, are similar to these. They lead to an understanding of the interaction of stimulants and herbicides with the metabolic products of the plant itself. It creates the prerequisites for a guided influence in plant metabolism by physiologically active preparations.

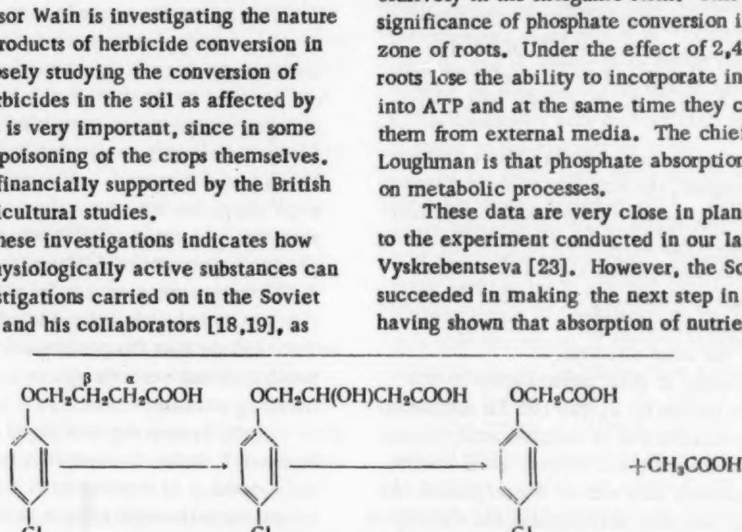
A great deal of attention is paid to the mechanism of herbicide activity in the agricultural department of Oxford university. Under the leadership of Professor G. Blackman, a study is being conducted there of the penetration and movement of herbicides when introduced through roots. It was found particularly that, on poisoning root respiration, the absorption of herbicides and their transport into other organs is greatly retarded. This indicates the necessity of metabolic energy for the stated processes.

The Oxford agronomy department is little concerned with practical problems of agriculture, although it owns an experimental station of 160 acres of soil and a forest mountain range near the city.

The main studies here are of a theoretical nature. Especial attention is devoted to metabolism of roots in relation to their absorptive activity.

B. Loughman [21,22] accomplished an interesting study here on the initial stages of inorganic phosphate absorption by barley roots. Using P^{32}-labeled phosphate Loughman showed that even in the first 15-30 seconds the absorbed phosphorus enters the nucleotides in considerable amounts-chiefly into ATP and ADP. A few minutes later the labeled phosphorus is also found in the hexosephosphates. Despite the rapid inclusion of inorganic phosphate into the root metabolism, the transition of phosphorus into the shoots is accomplished almost exclusively in the inorganic form. This indicates the local significance of phosphate conversion in the absorptive zone of roots. Under the effect of 2,4-dinitrophenol, roots lose the ability to incorporate inorganic phosphates into ATP and at the same time they cease to absorb them from external media. The chief conclusion by Loughman is that phosphate absorption by roots is based on metabolic processes.

These data are very close in planning and results to the experiment conducted in our laboratory by É. Vyskrebentseva [23]. However, the Soviet investigator succeeded in making the next step in this problem, having shown that absorption of nutrient salts by squash



4-Cl-phenoxybutyric acid — 3-Cl-phenoxy-β-hydroxybutyric acid — 4-Cl-phenoxy-acetic acid

roots is accompanied by loss of nucleotides, i. e., that root nutrition is accomplished by use of energy of ATP phosphate bonds.

J. Harley and his collaborators are studying root nutrition of woody plants by use of mycorhizae. In this connection, a detailed study was made of the respiratory and oxidative enzymes on mycorhizal fungi symbiotic with beech roots [24, 25].

These investigations showed that absorption of phosphates by mycorhizal fungi as well as by plant roots is accompanied by rapid esterification and activation of respiration. Using P^{32} it was shown that with the participation of mycorhizae the roots of Fagus sylvatica absorb inorganic phosphate 4 times as fast as without it. At first the greater part of the absorbed phosphate is concentrated in the fungal mycelium and only later is transferred to the roots of the host plant. This process of transmission requires a temperature higher than 10° and the presence of oxygen. In addition, the fungus must have a constant sugar supply. Harley and Jennings [26] have shown that beech mycorhizae absorb glucose more easily than they do fructose. Sugar absorption is accomplished by an accelerated respiration similar to one observed in salt absorption. This result leads the authors to conclude that intake of hexoses into mycorhizae occurs with the consumption of energy-rich phosphate bonds.

Interesting data were also obtained recently by Harley regarding potassium absorption by beech roots as aided by mycorhizae. According to his data the optimum K absorption occurs in this plant at about 15°. At 25° and at temperatures below 5° excretion into the external medium begins. Thus, potassium nourishment of beech can be accomplished only in a narrow temperature range, evidently as a result of special symbiotic relationships between the fungus and roots of the host. Since the physiology of root feeding of mycorhizal plants has undergone little study so far, and since its elucidation is of great value to forestry, the studies by Harley are very notable indeed.

In the botany department of Oxford University presided over by Darlington, the structure of waxy coatings on leaves of different plants is being studied by B. Juniper by electron microscopy [27]. These studies have a practical value since they permit evaluation of leaf moistening and their accessibility to nonroot feeding, to chemical preparations and other activities by the ultra-structures of the outer coatings.

Of supreme interest to plant physiologists in this department are the studies by V. Butt and his collaborators devoted to the distribution of ascorbic acid oxidase and some other enzymes in plant tissues. As is known, the oxidation of ascorbic acid can be accomplished not only by the specific ascorbic acid oxidase but directly or indirectly by cytochrome oxidase, peroxidase, polyphenol oxidase, copper ions and some other factors. Therefore, initially V. Butt paid much attention to proving the presence of ascorbic acid oxidase in plant tissues. For this purpose he used chiefly procedures of specific retardation of this enzyme, inhibition of accompanying oxidases and specificity to different substances. As a consequence, the widespread prevalence of ascorbic acid oxidase in higher plants was found [28,29].

Subsequently, in studying roots of barley and peas, the Oxford physiologists found that the major portion of ascorbic acid oxidase is firmly fixed on cellulose membranes from which this enzyme can not be removed either by mechanical or physicochemical means. At the same time it was shown that in endings of growing rootlets as well as in the zones of expanding stems up to 40% of ascorbic acid oxidase is still in soluble form and that only later does it almost fully combine with the cellulose membrane [30]. The authors assume that chemical bonds form with -OH groups of the cellulose [31].

In this connection it is interesting to note that in the Soviet Union M. Bokuchava [32] found that in tea leaves two oxidative enzymes – polyphenol oxidase and peroxidase – can also be firmly bound with cell walls.

Besides oxidative enzymes, other enzymes can also be fixed on cell walls. For instance, as the latest studies by Butt show, invertase and phosphatase are fixed in a similar manner.

All these data indicate that cell membranes are not mechanical elements, but are alloted various enzymatic functions, at least during a given period of time. This conclusion is important in understanding the picture of inner growth; as applied to roots, it is of interest from the point of view of their absorbing function, to which the studies of E. Ratner [33] and some others are dedicated in the Soviet Union.

Finally, studies on reduction of nitrates and nitrites in plants conducted in Butt's laboratories should be mentioned. According to theories developed here, the reduction of TPN, by which the reduction of oxidized nitrogen compounds occurs, is not accomplished in the process of ordinary respiration, but in the pentose cycle. This is proven by the fact that in the presence of glucose-1-C^{14} the reduction of nitrates is accompanied by considerable liberation of $C^{14}O_2$ (conversion of glucose-16-phosphate into ribose-5-phosphate), while with glucose-6-C^{14} the process proceeds for a long time with liberation of labeled carbon dioxide. On this basis the assumption is made that the pentose cycle in plants plays not so much a general energy role as a specialized function in reducing nitrates.

In the botany department of Cambridge University Professor J. Barker is occupied, as formerly, in studying the chemistry of respiration in higher plants (potato tubers, apples) with oxygen variation. In recent years he found a change in respiratory systems when the atmosphere is enriched by oxygen up to 50%. In such cases the cytochrome oxidase system is activated and the ascor-

bic acid content is increased. At present Barker is occupied with a study of phosphorus metabolism in tissues respiring at a low partial O_2 pressure. These investigations are similar to those conducted at the K. A. Timiryazev Institute of plant physiology on root metabolism under conditions of an insufficient oxygen supply.

In the same laboratory M. Canny is studying transport of substances in plants. Combining the isotope method with Mittler's technique (generation of phloem exudates through aphid stings) Canny has shown that the radioactive assimilants in exudates of white willow are represented almost exclusively by sucrose during the first hours of feeding the leaf $C^{14}O_2$. This confirms the assumption of the dominant role of sucrose in transport mechanism. Canny himself considers this process a result of metabolism in conducting tissues. By using radioactive assimilants moving strictly along phloem elements, he succeeded in measuring the phloem respiration (by the liberation of $C^{14}O_2$) in grapevine leaf petioles not detached from the plant. In this case Canny found that the conducting tissues truly show a very intense respiration. The high respiratory activity of conducting cells was initially found by M. Turkina [34] and then was confirmed on numerous occasions. This was considered in relation to the function of substance transport, and thus it favored the metabolic nature of this phenomenon. However, it is still possible that the increased gas metabolism in the vascular-fibrous clusters extracted from the plant could have been caused by their injury or by easier access of oxygen. This question was difficult to resolve experimentally, and Canny deserves merit for his success in doing it.

Considerable attention is being given in Cambridge University to the study of plant growth under controlled environments of illumination, temperature and moisture. These studies are conducted with the closest cooperation of Professor G. Briggs. The British physiologists seek a technical solution of this problem not by constructing large installations of the phytotron type, but by building small portable chambers (for instance, 1.1 by 0.65 by 0.85 m), which are inexpensive and are easily suited to accomodation in relation to desired conditions: see G. Evans [35]; A. Hughes [36]. Incidentally, it should be noted here that the climatic compartments for plant experimentation being built at present at the Rothamsted experimental station are also rather modest in their dimensions, although they are more spacious than the chambers used in Cambridge.

Of considerable interest to plant physiologists studying polyphenols are the studies in the Low Temperature Station in Cambridge. This institution is financed by the Royal Society for Agricultural Experimentation, although in its activities it is closely associated with the university. The chief problem consists in solving practical problems of preserving products of animal and vegetable origin at low temperatures. However, in addition many theoretical investigations are being conducted here, chiefly on leuco-anthocyans, flavones, coumarins and other polyphenols of vegetable origin.

Methods of chromatographic separation of the phenol derivatives are well defined here, and the composition of polyphenols in plants of various classification groups is throughly investigated by E. Bate Smith [37]. Swen relates the physiological activity of catechins to their stereo configuration. Since they have glucosidic linkages, catechins and other polyphenols lose their physiological activity and acquire mobility in plants with stereo-configuration changes. This last assumption is presently being investigated by Swen with the aid of the Mittler method (aphids).

In London, in the Imperial College of Science and Technology the department of plant physiology has been headed in recent years by Prof. W. James, who is well known to Soviet plant physiologists by his investigation of oxidation conversion in plants, as well as by his book, "Plant Respiration," translated into Russian in 1958.

Not long ago W. James and W. Slater [38] conducted an investigation on conversion of pyruvate (labeled by C^{14} in carbonyl) in barley rootlets and some other tissues. They showed that in this case the pyruvate is very rapidly converted to alanine, which possibly indicated its direct amination. Moreover, pyruvate participates in the formation of malate, citrate and auxinate, which conclusively indicates functioning of the Krebs cycle in roots. These results coincide well with the studies of Soviet scientists devoted to the clarification of the internal mechanism of the absorption activity of roots [39].

Professors H. Porter and I. Edelman and their collaborators in the same department, known mainly for their studies on carbohydrate metabolism, are now investigating sugar transport. Particularly H. Jones, R. Martin, and H. Porter have shown that labeled sucrose formed in tobacco leaves during photosynthesis with $C^{14}O_2$ disappears very rapidly from the mesophyll and is found in the conducting paths.

At the same time other sugars remain in the leaf for a prolonged period of time. Thus, these results once again indicate the exceptional role of sucrose in transport of organic substances. Subsequent studies by the same authors made possible clarification of the distribution picture of assimilants in the intact plant. In this case the results of investigations on tobacco by British scientists generally came quite close to results on soya found by Belikov [41] in the Soviet Union.

The Imperial College scientists consider the transport of organic substances to be a metabolic process, in accordance with the views of our laboratory. In particular, Edelman expresses an original point of view of the mechanism of sugar transport as something in the nature of a chain reaction of transfructosidation in which the sucrose molecules are not transmitted per se, but serve only as intermediate acceptors of fructose residues

carried over by enzymatic paths. This interesting view, however, requires experimental verification.

The experimental station of the Imperial College—Sylwood Park—is located 25-30 miles from London. The station has a laboratory building, a library, hothouses and has close to 50 hectares of ground occupied by the park and field sectors.

Sylwood Park is a base for students' practice and simultaneously for scientific studies. A great deal of attention is paid here to studies of sick plants. In particular C. Batts and his co-workers are occupied with the study of two important grain pests—uredinales [42] and ustilagineae [43-45].

Another notable center in London where studies on plant physiology are conducted is the laboratory of Prof. G. Bennett-Clark in the botanical department of Kings College (London University). One of the directions developed here by Dr. D. Davies consists in a study in biosyntheses and conversions of organic acids and amino acids. As far back as 1952 Davies was one of the first to offer weighty evidence favoring functioning of the Krebs cycle in plants [46]. Later he found and studied enzymes of the three-carbon cycle in mitochondria of peas; this indicates participation of the Krebs cycle in plant respiration [47,48]. However, the portion of the cyclophorase system participating in the general respiration of growing tissue is not clear, in his opinion, and this requires special investigation [49]. At present Davies and his co-workers are busy studying the biosyntheses and interconversion of amino acids. In particular, they showed that serine can form in pea sprouts, as in animal tissues, from phosphoglyceric acid through phosphoserine [50]. A separate study was made of possible paths of interconversion of aspartic acid, serine and glycine in plants [51]. Acknowledging the multiplicity of paths in biosynthesis of acids and amino acids, Davies assumes that the initial assimilation of ammonia is accomplished in plants by only one path—through α-ketoglutaric acid.

Also Dr. J. Sutcliff, who for a number of years has been studying the absorption of ions by plant tissues [52,53], is conducting interesting investigations in Kings College. His main conclusion is that ion absorption is accomplished through metabolism, the energy of which is used for the labile bonding of ions entering the cell, and also for their transport through the protoplasm and for liberation into the vacuole. However, Sutcliff sees no direct relation between respiration and salt absorption. In his opinion the process of labile ion bonding which precedes their transport and a deeper involvement in metabolism is closely related to the biosynthesis of proteins. Indeed, when protein synthesis was inhibited in plant tissues by use of chloramphenicol, Sutcliff observed a stoppage of ion absorption, although respiration was not affected by it [54].

Bennett-Clark [55] assumes another possibility of labile bonding of anions and cations in their absorption. According to his theory, the initial fixation of ions is accomplished on amphoteric phosphatides (for example, on lecithin) in their enzymatic conversions proceeding in a closed cycle. As far as the active transport of ions through the protoplasm is concerned this process is accomplished through energy of phosphate bonds, in the opinion of Kings College physiologists.

Subsequent investigations will probably be directed to growing cells in search of substances—acceptors and conductors—responsible for the first stages of absorption and accumulation of ions.

All these studies are of great interest to Soviet plant physiologists, who also pay serious attention to metabolic processes related to the initial assimilation of ions.

Interesting studies on plant physiology are being conducted in Scotland. In the present review we will touch only on some studies conducted under the leadership of Prof. R. Brown in the botanical department of Edinburgh University.

The attention of this laboratory is centered on studying the physiology of plant growth. Prof. Brown considers that interest in auxins and, more recently, also in gibberellins to a certain extent retarded the progress of growth physiology proper. This section should begin with a systematic study of the metabolism of growing cells, in which auxins as well as many other substances should occupy their place. The investigation on growth is conducted in Brown's laboratory on tips of pea rootlets, which are divided into sections 1.5-2.5 mm long, corresponding to the three chief zones of growth. The study is so organized that each co-worker studies a separate phase of the general problem (the amino acid composition, enzyme activity, respiration, nucleic acids, rate of growth and cell fission, etc.). Obviously, such a study can lead to very important and broad conclusions, but even at this time certain interesting results can be mentioned.

By tissue maceration with 5% chromic acid, the investigators counted the number and dimensions of cells at the tips of rootlets taken at different periods. These counts showed that the cell growth in the meristem proceeds approximately along the same curve as does the growth of organisms in a culture.

In another investigation it was shown that the uppermost meristem of rootlets and stem growth points is distinguished by a relatively low metabolism. This results from the fact that the ratio of enzymatic activity to the sum of proteins in cells in the fission stage is lower than in other zones of growth. Evidently, in the first stages of cell formation the major portion of proteins have not become enzymes as yet; this occurs later, as the cells expand and form vacuoles.

In the growth process, especially in the stage of expansion, there must occur a sliding of tissues or a displacement of cells in relation to one another. An examination of this process has shown that the stability of bonds between cells is controlled by the enzyme appara-

tus. It is possible that the decisive role belongs to the oxidative enzymes in this case since it was noted that in the presence of O_2 the meristem cells are replaced and dissociate more easily than in a nitrogen atmosphere. Thus, as Butt does at Oxford (see page 624), Brown also concludes that the cell walls, or, to be more specific, the proteins fixed on them, possess a high enzymatic activity.

Of interest also are the experiments which show that ribonuclease introduced into the culture medium arrests the mitoses in the uppermost meristem of rootlets, which is evidently related to inhibition of protein synthesis.

The enumerated examples do not exhaust all the studies conducted in Edinburgh on plant physiology; however, they illustrate the approach to the problem, and we believe that the path selected by Brown and his co-workers in the study of growth deserves serious attention of the Soviet plant physiologists.

This review does not pretend to be complete. The author's brief visit to Great Britain did not allow him time for details of numerous other studies. However, even from this short acquaintance a conviction arises that problems of plant physiology are studied intensely and successfully in Great Britain. This science occupies a position of great importance in the cycle of biological disciplines and exerts a growing influence on the theoretical foundations of agriculture.

In conclusion the author desires to gratefully acknowledge the invariable courtesy he encountered and ready disposition to share the results on the part of the scientists whose laboratories in Britain and Scotland he visited. The British Council on scientific and cultural relations graciously extended its aid in organizing travel and accomodations throughout England. The assistance of the Royal Society was especially valuable; by its influence it created the best possible environment for this study.

The author was pleased to find in the membership records of the Royal Society in London the name of K. A. Timiryazev, who was elected to membership of this old academy on November 9, 1911.

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* See English translation.

MISSING PAGES ARE INDEX PAGES
WHICH HAVE BEEN PHOTOGRAPHED
AT THE BEGINNING OF THE VOLUME(S)

ERRATA

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RUSSIAN JOURNALS FREQUENTLY CITED
[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiya	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiya	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Émbriol.	Arkhiv Anatomii Gistologii i Émbriologii	Archives of Antomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiya	Biokhimiya	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiya Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Byull. Éksptl. Biol. i Med.	Byulleten Éksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Byull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Byulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Éksptl. Khirurg.	Éksperimentalnaya Khirurgiya	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiya i Toksikologiya	Pharmacology and Toxicology
Farmatsiya	Farmatsiya	Pharmacy
Fiziol. Rastenii	Fiziologiya Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenov Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariya	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiya Tikhookeanskogo N. I. Instituta Rybnogo Khozyaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiya	Khirurgiya	Surgery
Klin. Med.	Klinicheskaya Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo (po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaya Parazitologiya i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaya Radiologiya	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiya	Mikrobiologiya	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhyat. i Psikhogig.	Nevropatologiya, Psikhyatriya i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediya, Travmatologiya i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriya	Pediatriya	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Éndokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniya Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaya Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journal
Stomatologiya	Stomatologiya	Stomatology

* BRITISH-AMERICAN TRANSLITERATION SYSTEM.

Abbreviation	Journal	Translation
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriya	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiya	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiya	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiya, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoyuz. Gidrobiol. Obshchestva	Trudy Vsesoyuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoyuz. Inst. Ekspti. Med.	Trudy Vsesoyuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiya	Urologiya	Urology
Uspekhi Biokhimiya	Uspekhi Biokhimiya	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiya	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriya Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universitera, Seriya Biologii i Pochvovedeniya	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiya	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariya	Veterinariya	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniya	Voprosy Pitaniya	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaya Laboratoriya	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiya	Journal of General Biology
Zhur. Vysshei Nerv. Deyatel.	Zhurnal Vysshei Nervnoi Deyatelnosti imeni L. P. Pavlova	L. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci. Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.